

## Changes in Activities of the Acid and Alkaline Phosphatases during the Metamorphosis of the Pine Moth, *Dendrolimus spectabilis* Butler

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솔나방의 變態에 따른 Acid, Alkaline Phosphatases의 變化

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### 摘 要

솔나방(*Dendrolimus spectabilis*)의 變態에 따른 acid 및 alkaline phosphatase의 活性度の 變化를 測定하였다.

두 酵素의 活性도는 幼虫의 成長과 더불어 增加하고 前蛹期에서 減少하였다. Acid phosphatase는 蛹期の 初에, 그리고 alkaline phosphatase는 幼虫8齡에서 각각 最高의 活性를 나타내었다. 前蛹期에서 두 酵素간의 活性度の 차이는 크게 나타나지 않았으나 幼虫8齡에서 보다는 훨씬 낮았다.

Acid phosphatase의 活性도는 蛹期初에 增加하고 蛹期末에서 減少하다가 成虫에서 다시 上昇하였다.

Total phosphatase의 活性度の 變化는 變態에 따른 生理的曲線인 U字型 pattern을 나타내었다.

### INTRODUCTION

The physiological functions of the phosphatases in insect are not as well understood as those of other enzymes. But they have shown to be important in the metabolism of the carbohydrates, nucleotides and phospholipids (Lambremont, 1960). Phosphatases apparently bring about a linkage of lipid-carbohydrate synthesis and intraconvertibility, and play a part in the processes of muscle contraction. Moog (1964) summarized the roles of alkaline phosphomonoesterase in both vertebrate and invertebrate animals to include synthesis, tissue growth and differentiation, and synthesis of nucleic acid and protein including silk proteins.

Other workers have shown that the phosphatases act in the active transport of materials across a membrane barrier. Vermehren (1939) showed that the level of phosphatases in human blood serum was characteristics of age and of the general metabolic and pathologic state, and alkaline phosphatase enzyme decreased with increasing age, but was also influenced by diet, and Zorzoli (1955) reported that acid phosphatase of mouse liver remained constant in young and adults, then sharply decreased in senile mice, although a concurrent rise in alkaline phosphatase took place. Rockstein (1950) was one of the first to study enzyme changes in insects. In 1953, he studied glycerophosphatase in the adult worker honey bee and found that a 90% rise in activity occurred up to the 10th day, with no changes thereafter, while alkaline phosphatase decreased to about 44% below that of day-old bees by the 10th day, with no further changes.

Later Rockstein (1956) found that the activities of both acid glycerophosphatase and magnesium-activated ATP-ase of the house fly declined gradually with age to a minimum at about the 11th day, and the power of flight was lost by the end of the 14th day. He suggested that the reduction in the ability of the cell to metabolize phosphorus might be responsible for the decline of flight ability in the male house fly, and could be, in effect, a physiological sign of senescence.

Drilhon and Susnel (1945) demonstrated the presence of a phosphatase with an alkaline pH optimum in the gut, and one with an acid pH optimum in the Malpighian tubes of the insect. An active acid phosphatase has been found by Denuce (1952) in the silk gland of *Bombyx mori*,

Barker and Alexander (1958) found decreases in activity of acid and alkaline phosphatases in homogenates of the adult house fly.

Recently, Lambremot (1960) studied the postemergence changes of enzyme activity in the mosquito, *Aedes aegypti* and found that acid phosphatase was at its maximum in both sexes of mosquito during the first day of adult life.

The present paper deals with comparisons of the activities of acid and alkaline phosphatases during the growth and metamorphosis of the pine moth *Dendrolimus spectabilis* Butler.

## MATERIALS AND METHODS

The larvae of the pine moth, *Dendrolimus spectabilis*, were collected and reared on pine needles in a breeding box at 25°C until used for experiments. The larvae were examined daily and the insects were taken from the pine needles at the desired age.

Acid and alkaline phosphatase activities were measured on 5% homogenates according to the method of Bodansky (1933) using sodium beta-glycerophosphate as

the substrate. The insects were homogenized in a motor-driven homogenizer for 5 minutes in ice-cold distilled water and centrifuged. The supernatant was obtained and made up to a known volume and then used as the enzyme extract.

Acid phosphatase was measured at a pH of 5.0 and alkaline phosphatase at 9.8 respectively. Homogenates were incubated with substrate for 30 minutes in a water bath at 35°C. Released orthophosphate was determined spectrophotometrically on a Bausch and Lomb Spectronic 200 at 720 millimicrons.

Final activity is expressed as micrograms of phosphorus released per ml of homogenate per 30 minutes of reaction time. Each value is an average of at least five determinations.

## RESULTS

The activity of acid and alkaline phosphatase at representative metamorphosis stages in the pine moth, *Dendrolimus spectabilis*, is shown in Table 1.

The activities of acid and alkaline phosphatase generally increase with the growth of larvae and, in the 8th instar larval stage, the activity of alkaline phosphatase is by far higher than that of acid phosphatase. And in the prepupal stage there are no significant differences between acid and alkaline phosphatases.

**Table 1.** The activity of acid and alkaline phosphatases of the pine moth, *Dendrolimus spectabilis* Butler.

Stage	Acid phosphatase	Alkaline phosphatase ( $\mu\text{g}$ phosphorus/g protein)	Total phosphatase
6th instar larva	0.40	1.21	1.61
7th instar larva	0.56	1.56	2.12
8th instar larva	1.40	4.27	5.67
Prepupa, 1 day	1.24	1.27	2.51
Pupa, 1 day	3.31	1.07	2.48
Pupa, 3 day	1.32	0.62	1.94
Adult, 1 day male	2.25	1.01	3.26
female	2.46	1.09	3.55

However, from Table 1, it can be observed that their activities are by far lower than in the 8th instar larva.

In the pupal early stage there occurs an increase in the activity of total phosphatase followed by a decrease in the pupal later stage, and in the adults stage their values increased again. And both enzyme activities of adult are somewhat higher in female than in male.

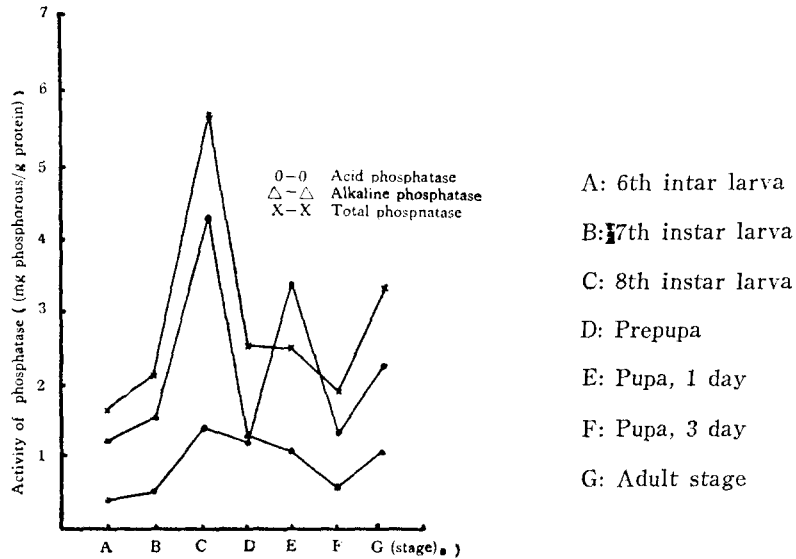


Fig. 1. Activities of acid and alkaline phosphatases of pine moth, *Dendrolimus spectabilis* Butler.

The total phosphatase activity also increases steadily during the larval stages, decreases in the prepupal stage, again increasing in the adult stage.

## DISCUSSION

The activity of phosphatases in this experiment generally agrees with the results of other investigators (Ludwig, 1962; Sridhara, 1963).

Sugar phosphates are probably normal constituents of insect hemolymph, although the presence of active phosphatases has probably interfered with their measurement in some cases.

Phosphomonoesterases of undetermined specificity have been recorded in insects by several workers, using biochemical and histological methods. Drilhon and Busnel (1945) reported the presence of a phosphatase with an alkaline pH optimum in the gut, and one with an acid pH optimum in the Malpighian tubes of the insect.

An active acid phosphatase has been found by Denuce (1952) in the silk gland of *Bombyx mori*. Faulkner (1955) has demonstrated the presence in silkworm blood of a specific hexose-1-phosphatase, with an acid pH optimum in the range 4.0-4.5. This enzyme hydrolyzes glucose-1-phosphate, galactose-1-phosphate, and p-nitrophenyl phosphate at an appreciable rate, having little or no action on

hexose-6-phosphates and ribose or triose phosphates.

Although phosphomonoesterases have been identified in insect tissues of all kinds, the specificity of these enzymes has received little attention. It is known that silkworm hemolymph contains a specific glucose-1-phosphatase which is quite inactive against glucose-6-phosphate, and in fact, the hemolymph of this species contains an appreciable amount of glucose-1-phosphate. The route from glycogen to glucose is thus shorter by one step than it is in mammals, which possess a glucose-6-phosphatase.

The activities of both acid and alkaline phosphatase increased with the growth of larva. The increase in their activities agrees with the results of Sridhara and Bhat (1963) for the silkworm, *Bombyx mori*. Sridhara and Bhat (1963) reported that increase in the activity of the enzymes during larval development was reflected in a decrease in the acid soluble phosphorus content.

Barker and Alexander (1958) and Ashrafi and Fisk (1961) reported that the maximum activities for both the enzymes were shown in the first egg stage and in the pupal stage, respectively.

The activity of phosphatases in the egg stage was not measured here. Whether the activity of enzymes is in maximum in the first egg stage can not be decided without further study on the egg. But alkaline phosphatase only appears the highest activity in the 8th instar larva. It seems likely that these differences are of physiological characteristics of different groups of insects.

Sridhara and Bhat (1963) reported that during the pupal stage the alkaline phosphatase was almost absent, whereas the acid phosphatase maintained a high and constant value.

As may be seen from Table 1, the activity of alkaline phosphatase during the pupal stage is far lower than that of the acid phosphatase. The highest increase of the alkaline phosphatase during the 8th instar larva [and its sudden decrease seems to be an important role in the metabolism of carbohydrates and fatty acids. The reason for this might be ascribed to the fact that it is only during the 8th instar that larvae consume their pine needles voraciously and need mechanism for the steady transport of the metabolites across the intestinal wall.

Sridhara and Bhat (1963) had also obtained the same result. Barker and Alexander (1958) reported that phosphatase activity was somewhat higher in female than in male of house fly. According to Table 1, it seems that such a phenomena may be operative here.

The observed behaviour of alkaline phosphatase during moulting would also suggest the possibility of a direct or indirect hormonal control of its activity.

### SUMMARY

The activity of acid and alkaline phosphatases of pine moth, *Dendrolimus spectabilis* Butler was measured in a series of developmental stages ranging from the larva to the adult. The activity of both enzymes increased gradually with age of larvae, and then decreased in the prepupal stage.

Acid enzyme was at a maximum in the pupal early stage and alkaline enzyme in the 8th instar larva, respectively. And in the prepupal stage there were no significant differences between both acid and alkaline phosphatases.

However, their activities were far lower than in the 8th instar larva. In the pupal early stage there occurs a increase in the activity of acid enzyme followed by a decrease in the pupal later stage, and in the adult stage its activity increased again.

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