# Acid and Alkaline Phosphatases of the Pine Moth, Dendrolimus Spectabilis Butler

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개 요

松虫의 acid 및 alkaline Phosphatase의 活性度를 발생단계인 幼虫에서 부터 成虫에 이르기까지 測定하였다. 兩酵素의 活性度는 幼虫의 成長과 더불어 점점 增加하고 前蛹期에 감소하였다. Acid phosphatase는 蛹期初에 그리고 Alkaline phosphatase는 幼虫齡에서 各各 最高의 값을 나타내었다. 그리고 前蛹期에서 兩酵素間의 差異는 별로 없으나 幼虫齡에 있어서 보다는 훨씬 높았다. 蛹期初에 兩酵素의 活性度는 증가하고 後期에 감소, 成虫時期에 다시 上昇하였다.

## Abstract

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 the enzymes increased gradually with age of larvae, and in the prepupal stage, it decreased. 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 alkaline phophatases. 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 both enzymes followed by a decrease in the pupal later stage, and in the adult stage their activities increased again.

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## Introduction

The physiological functions of the phosphatases are not as well understood as those of other enzymes. But they have shown to be important in the metabolism of carbohy-drates, neucleotides, and phospholipids (Lambremont, 1960). Phosphatases apparently bring about a linkage of lipid-car-bohydrate synthesis and intraconvertability, and play a part in the processes of muscle contraction. Moog (1946) summarized the roles of alkaline phosphomonoesterase in both vertebrate and invertebrate animals to include synthesis of cuticle, 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 acid glycerophosphatase in the adult worker honey bee and found that a 90% rise in activity occurred up to the 10th day, with no changes there after, 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 ll 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 sensescence. Barker and Alexander (1958) found decreases in activity of acid and alkaline phosphatases in homoginates of the adult house fly. Recently Lambremont (1960) studied the postemergence changes of enzyme activity in the mosquito 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 activites of acid and alkaline phosphatases during the growth and metamorphosis of pine moth, Dendrolimus spectabilis Butler.

## Materials and Methods

The larvae were collected and reared on pine needles in the laboratory (approximately 25°C). The larvae were examined daily and at the desired age, the insects were taken from

the pine needles. Acid and alkaline phosphatase activities were measured on 5% homoginates according to the method of Bodansky (1933) using soidum beta-glycerophosphate as the substrate. The insects were homoginized 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. Homoginates were incubated with the substrate for 30 minutes in a water bath at 35°C.. Released orthophosphate was determined spectrophotometrically on a Bausch and Lamb Spectronic 20 at 720 millimicrons. Final activity is expressed as micrograms of phosphorus released per ml of homoginate per 30 minutes of reaction time.

Each value is an average of at least 5 determinations.

## Results

The activity of acid and alkaline phosphatase are given in Table. I.

**Table I. Acid and alkaline phosphatases of the pine moth, Dendrolimus spectabilis Butler**

| Stages | Acid Phosphatase | Alkaline Phosphatase | 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 |
| Prepura, 1 day | 1.20 | 1.27 | 2.47 |
| Pupa, 1 day | 3.31 | 1.07 | 2.48 |
| Pupa, 3 days | 1.32 | 0.62 | 1.94 |
| Adult, 1 days | Male 2.25 | 1.01 | 3.26 |
| | Female 2.46 | 1.09 | 3.55 |

The activities of acid and alkaline phosphatase generally increases 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 both acid an alkaline phosphatases. However, from Table I, it can be observed that their activities are by far lower than in the 8th instar larva. In the pupal early stage there occurs a increase in the activity of both enzymes followed by a decrease in the pupal later stage, and in the adult stage their values increased again. And both enzyme activities are somewhat higher in female than in male of adult. The total phosphatase activity also increases steadily during the larval stages and decreases in the prepupal stage, again increasing in the pupal early stage, and in the pupal later stage its activity decreased, and then

increased again in the adult stage.

## Discussions

The activity of phosphatases in this experiment generally agrees with the results of other investigators (Ludwig, 1962; Sridhara, 1963). The activities of both acid and alkaline phosphatase increased with the growth of larva. This increase in their activities agrees with the result of Sridhara and Bhat (1963) for the silkworm, Bombyx mori.

Sridhara et al (1963) reported in their study that increase of the activity of the enzymes during larval development was reflected in a decrease in the acid soluble phosphours content. Barker et al (1953) and Aschrafi et al (1961) showed that they observed maximum activity for both the enzymes in the first egg stage and secondly in the pupal stage, respectively. The activity of phosphatases in the egg stage was not measured here, whereas 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 that these differences are physiological characteristics of different groups of insects. Sridhara et al (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 I, 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 inster larva and its sudden decrease seems to be an important role in the metabolim of carbohydrates and fats. For 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 et al (1963) had also obtained the same result in their study. And Barker (1958) reported that phosphatase activity was somewhat higher in female than in male of house fly. According to the above Table I, it seems that such a phenomena may be operative here.

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