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Photoperiodic effect on some enzymes and metabolites in diapausing *Antheraea mylitta* pupae and *Philosamia ricini* larvae during development

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Abstract. The variation in acid phosphatase (EC 3.1.3.2) activity in *Antheraea mylitta* was similar in all light and dark groups exposed to different photophases (LD =0:24, 24:0, 18:6, 14:10, 10:14 and 12:12 h) maintaining all along a higher activity than its alkaline counterpart. The highest activity was recorded on day 82 in LD group 10:14 h. The non-diapausing *Philosamia ricini* larvae registered highest activity in LD group 0:24 h on day 5. Alkaline phosphatase (EC 3.1.3.1) activity was low all through metamorphosis in both the lepidopterans, although significantly elevated activity was observed on day 5 in larvae of all *Philosamia ricini* LD regimens and on day 82 in *Antherae mylitta.* Photoperiodic effect on Phosphorylase (EC 2.3.1.1) activity, glycogen and inorganic phosphates content have also been studied.

Exposure to LD 10:14, 14:10 and 18:6 h provoked early diapause termination in *Antheraea mylitta.* The non-diapausing *Philosamia ricini* was unaffected in moth emergence but the emerged adults of LD 24:0 and 0:24 h groups were unhealthy, small and did not mate or oviposit.

Keywords. Photoperiod; phosphomonoesterases; Phosphorylase; glycogen; *Antheraea mylitta; Philosamia ricini.*

Introduction

Several lepidopteran insects during their life cycle undergo a phase of diapause while confronted with unfavourable environmental conditions of temperature and day length. The effect of photoperiodism on enzymic activities and metabolites concentrations in developing insects when subjected to different photophases have not been investigated. In insects the presence of a number of phosphomonesterases of undetermined specificity has been detected by employing biochemical and histo-chemical techniques (Drilhon and Busnel, 1945; Denuce, 1958; Faulkner, 1955). Pant and Lacy (1969) suggested that acid phosphatase participates in glycogen degradation on the assumption that the high activity of the enzyme stimulates *Corpus cardiacum* hormone which by activating Phosphorylase induces glycogen degradation.

Some significant changes in amino-transferase activity, protein and total free amino acid content were observed when diapausing pupae of tasar silkworm *Antheraea mylitta* were exposed to different light and dark periods (Pant and Jaiswal, 1981). The present communication describes changes in the profile of active phophorylase, acid and alkaline phosphatases, inorganic phosphates and glycogen content in the fat body of *A. mylitta* diapausing pupa when subjected to different photoperiodic regimens of light and darkness, and compared with the changes occuring in the non-diapausing lepidopteran *Philosamia ricini* subjected to different photoperiodic exposures. *A. mylitta,* unlike *P. ricini* is a bivoltine lepdopteran undergoing a diapause for 180 days under adverse conditions of temperature while *P. ricini* is a non-diapausing multivoltine insect completing four to six cycles in a year.

Materials and methods

Larvae of *P. ricini* and diapausing cocoons of *A. mylitta* were reared and maintained under conditions as described earlier (Pant and Jaiswal, 1981). Three groups of 600 larvae of *P. ricini* at 4th instar stage were exposed to LD:24:0, 0:24 and 12:12 h. Assays were commenced after acclimatizing the insects to the experimental conditions of light-darkness exposures till they ecdysed to fifth instar stage. The light source employed in both insect experiments was 45 watt fluorescent tube.

Fat body homogenates (20% w/v) of both the insects at different developmental stages were prepared as previously described (Pant and Morris, 1969; Pant and Pandey, 1980). Three lots of six randomly selected pupae and larvae from each group were used. The strained individual and pooled homogenates of each group were assayed in triplicates and the average values were used to calculate standard deviations. Where the range was negligible no mention of any variation is made.

Acid (EC 3.1.3.2) and alkaline phosphatases (EC 3.1.3.1) were assayed as described earlier by Pant and Lacy (1969). Enzymic activity has been expressed as µg inorganic phosphate (P_i) liberated/mg protein/h. Inorganic phosphates were determined by Allen's method (1940) and expressed as mg phosphorus/g tissue fresh weight.

Phosphorylase (EC 2.4.1.1) activity was assayed by the method of Green and Stumpf (1942) as modified by Srivastava and Krishnan (1961). Enzymic activity was expressed as µg P_i/mg protein/30 min. Glycogen was isolated as described by Wiens and Gilbert, (1967) and determined by the method of Carrol *et al.* (1956) and expressed as mg glucose/g tissue fresh weight.

All estimations were made only upto day 131 since emergence commenced in one of the experimental insect groups thereafter.

Results and discussions

Light and dark period exposures of 18:6, 14:10 and 10:4 h enhanced emergence by days 45,33 and 27, respectively; while the group exposed to continuous light (LD= 24:0 h) emerged almost normally (day 182). However, in the insect group kept totally

unexposed to light (LD=0:24 h) emergence was delayed by 21 days (table 1). In LD groups 18:6, 14:10 and 10:14 h while moth emergence percentage was 80-95% in LD groups 24:0 and 0:24 h it was 67 and 31 % respectively with very high mortality rate in the latter group.

Table 1.

	Antheraea mylitta		*Philosamia ricini*	
L : D exposure (h)	Day of emergence	Adults % emerged	Day of emergence	% Adults emerged
12 : 12	179	98	18	90%
24 : 0	162	67	18	5%
0 : 24	200	32	18	25%
18 : 6	135	92	—	—
14 : 10	146	95	—	—
10 : 14	152	80	—	—

L : D = Light :dark.

Photoperiodic exposures appeared to have no effect on the non-diapausing *P. ricini* pupae in moth emergence. In LD 0:24 h group the pupae were shrivelled and very much shrunk in size and emergence was only 25%, while in LD 24:0 h group only 5% of the pupae emerged. The emerged adults of both groups appeared unhealthy and diminutive in size. Further, they did not mate or oviposit.

The acid and alkaline phosphatase activity of the fat body of the diapausing *A. mylitta* pupae exposed to different photoperiodic regimens varied similarly but the acid enzyme was present at a higher level all through development till emergence (figures 1Aa—Fa). The low activity present in almost all groups during days 54-68 increased on day 82,except in group LD 24:0 h (figure 1Ba) and reached the highest activity in group LD=10:14 h (figure 1 Da). Subsequently, the enzyme activity decreased and reached very low levels on day 96 in LD groups 10:14 and 14:10 h (figures 1 Da, Fa). On day 103 in LD 12:12, 24:0 and 0:24 h (figures 1 Aa, Ba, Ca) and on day 117 in LD 18:6 h (figure 1 Fa). With pupal development the activity also correspondingly increased till day 131 in all groups barring LD 10:14 and 14:10 h (figures 1 Da, Ea) where the activity got depleted.

Alkaline phosphatase on the other hand, as represented in figures 1Ab-Fb maintained a low level all through development and attained the highest activity in general, between days 82 and 96. However, in group LD 14:10 h although the enzyme revealed some activity on day 103 there was practically no activity during days 82-96.

The concentration of inorganic phosphate (figures 2Ac-Fc) varied more or less in a similar manner in all the 6 experimental insect groups. With an initial low concentration during early pupal development till day 75, they increased significantly between days 82 and 96, the maximum accumulation being in LD 0:24 and 12:12 h (figures 1Ac, Cc).

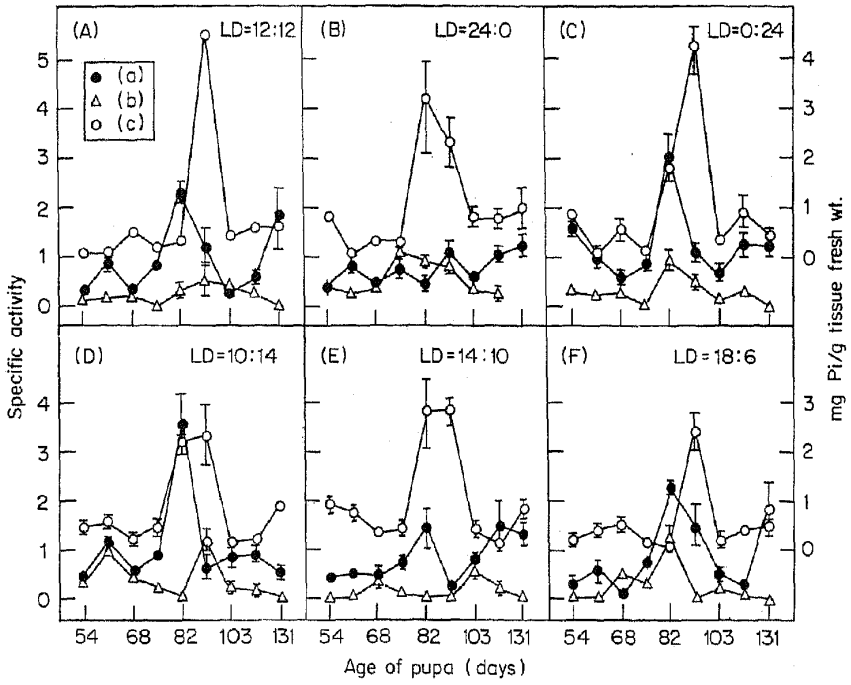

Figure 1. Alteration in the levels in acid phosphatase (●) alkaline phosphatase (Δ) and inorganic phosphates (O) in the fat body of the diapausing pupae of *A. mylitta* under different photoperiodic exposures.

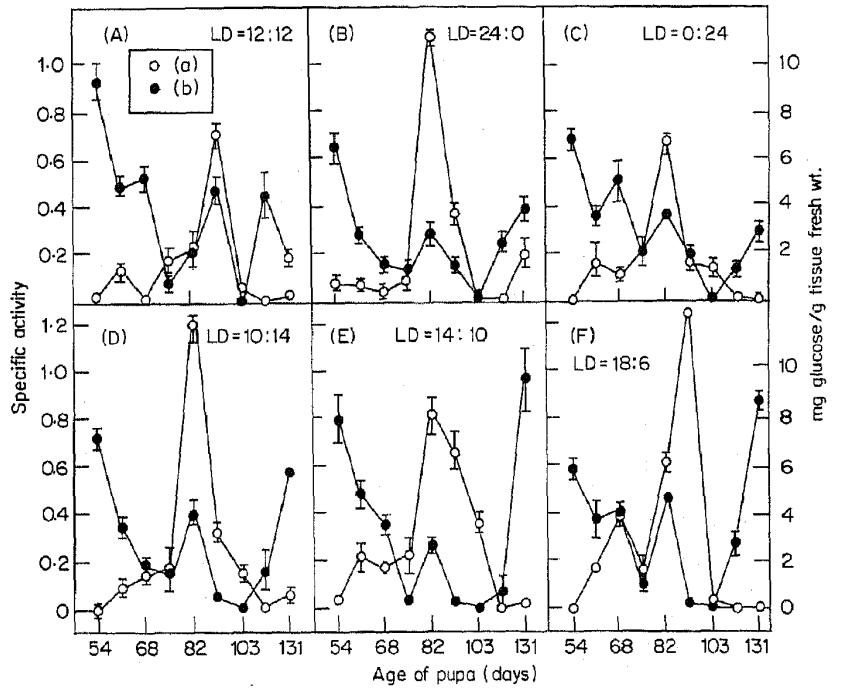

Figure 2. Phosphorylase activity (O) and glycogen (●) in the fat body of diapausing pupae of *A. mylitta* under different photoperiodic exposures.

In the non-diapausing *P. ricini* all the three exposures (LD 12:12, 24:0 and 0:24 h) revealed steady and regular increase in acid phosphatase activity each rise followed by an alternate low activity (figures 3Aa–Ca). The highest activity was recorded 0:24

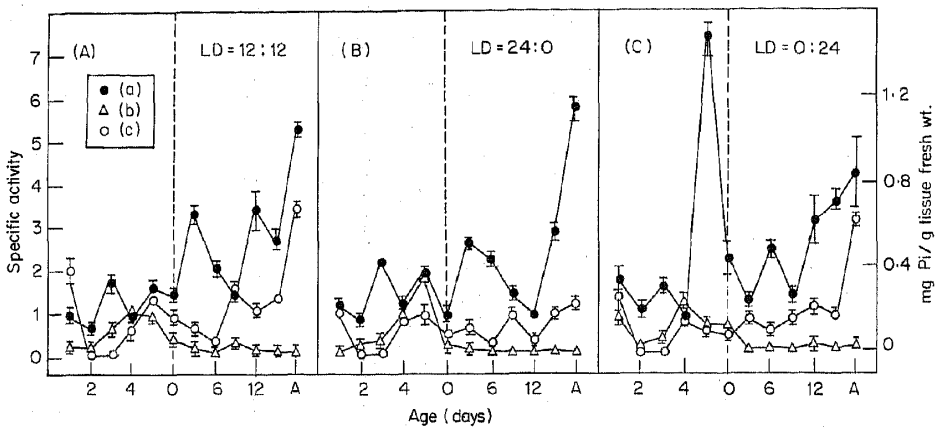

Figure 3. Changes in acid phosphatase (●) alkaline phosphtase (Δ) and inorganic phosphtates (O) in fat body of *P. ricini* under different photoperiodic exposure.

h on day 5 (figure 3 Ca) prior to commencement of spinning, the increase being seven-fold from ecdysis to fifth larval instar. However, on day zero pupa the activity was very low and interestingly, a pattern of alternating increase and decrease corresponding to that traced during larval development was observed. The net result was a two-fold increase in the activity till emergence. The LD 24:0 and 12:12 h varied in a similar manner as LD 0:24 h group without any enhanced activity. At emergence however, the activity increased several fold in both the regimens.

Alkaline phosphatase as in other insects, was maintained in all LD groups at a very low level all along larval and pupal development (figures 3 Ab-Cb). However, interestingly on day 5 prior to spinning, as in the case of acid phosphatase, the, enzymic activity showed a two-fold increase. During; pupal development however, the enzyme was practically inactive especially in LD 0:24 and LD 24:0 h groups (Figures 3 Cb, Bb).

Presence of appreciably high concentration of phosphates was detected in the control group in comparison with LD 0:24 and 24:0 h groups particularly on days 1, 4 and 5 in the developing larva and on pupal days 3 and 9. The newly emerged adults evinced the highest accumulation of phosphates (figures 3 Ac-Cc).

Phosphorylase activity of the diapausing *A. mylitta* pupae (figures 2 Aa-Fa) in all the groups was maintained at a very low level during early pupal period but with development the activity increased significantly, the maximum being recorded on day 82 especially in LD groups 18:6,10:14 and 24:0 h (figures 2 Fa, Da, Ba).Thereafter, the activity decreased in all the groups and reached a minimum value on day 103 and continued at this low level till day 131.

Likewise, *P.ricini* fat body also exhibited low Phosphorylase activity till day 3 in the 5th instar larva with a sudden elevation in its activity between days 4 and 5. The enzyme was maximally active in LD group 0:24 h on day 5 (figures 4 Aa-Ca).

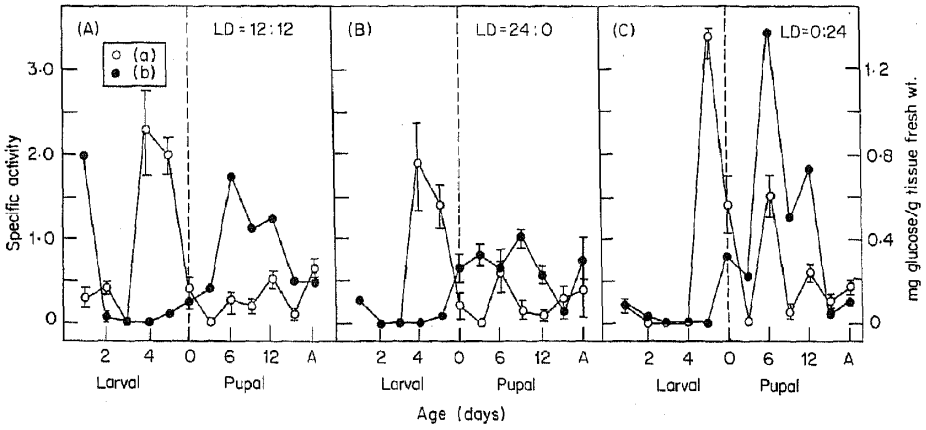

Figure 4. Variation in Phosphorylase activity (O) and glycogen content (●) in the fat body of *P. ricini* under different photoperiodic exposures.

Glycogen concentration on the other hand, as anticipated appears to be governed by Phosphorylase activity in both the lepidopterans. The high glycogen concentration observed in *A. mylitta* during early pupal development appeared to be utilized as noted from the gradual decrease thereof till day 75. This however, was slightly elevated on day 82 in LD groups 24:0,0:24,10:14,14:10,18:6 h (figures 2 Bb-Fb) and on day 96 in LD group· 12:12 h (figure 2 Ab). After declining markedly on day 103 in all experimental insect groups, glycogen steadily accumulated till day 131, the highest being was in group LD 14:10 h.

During 5th instar development the *P. ricini* fat body glycogen concentration(figures 4 Ab-Cb) varied inversely with Phosphorylase activity. With commencement of spinning, glycogen accumulation increased the maximum being in the 6 day old pupa. The all dark insect group (LD 0:24 h) concentrated higher amount of glycogen than LD groups 12:12 and 24:0 h.

The higher level of acid phosphatase compared to that of alkaline phosphatase activity at all stages during metamorphosis (Pant and Lacy, 1969), in adults (Sridhara and Bhat, 1963) and during embryonic development (Pant and Sharma, 1976) and its irregular pattern throughout the pupal period (Pant and Lacy, 1969) is a common phenomenon noted in several insects. Cori and Cori (1939) suggested that the occurrence of high acid phosphatase activity in the virtual absence of its alkaline counterpart was an adaptation to an active glycogen metabolism with a dephosphorylation in the acid range. In fact, in *P. ricini* pupae during development, a significant depletion of acid phosphatase was observed (Pant and Lacy, 1969). In addition, the present observations on the two lepidopteran insects *P. ricini* and *A. mylitta* subjected to different photoperiodic exposure further confirm this view.

One of the major carbohydrate reserves in insects' is glycogen which is stored mainly in the fat body and to some extent in muscles and other tissues, the enzyme phosphoryiase being one of the regulators of its synthesis and breakdown. This enzyme has been extensively studied by Cori and his collaborators (1943) who established its role in

glycogen synthesis. Phosphorylase breaks down glycogen to glucose and trehalose in order to provide energy for the vital processes like moulting, reproduction and flight etc. The *corpus cardiacum* hormone is believed to activate Phosphorylase and regulate glycogen levels. *A. mylitta* and *P. ricini* pupal fat body recorded high glycogen content during mid-pupal stage which in all probability is the remnant of the rich glycogen reserves accumulated towards the end of the voraciously feeding larval period. This high level declines steadily during pupal development when histolysis proceeds at a higher rate than histogenesis and practically disappears in the newly emerged adults. Bade and Wyatt's (1962) findings in *Cecropia* coupled with Pant and Lacy's (1969) for *P. ricini* further support the present observation.

With a concurrent depletion in glycogen content both the lepidopterans revealed high acid phosphatase activity. Since degradation of glycogen is believed to be under the hormonal control of *corpus cardiacum* (Steele, 1961) it is not unlikely that the high acid phosphtase activity observed in the developing pupa stimulates the *corpus cardiacum* hormone which in its turn enhances the Phosphorylase activity resulting in the degradation of glycogen.

In both the lepidopterans, despite the steady low maintenance of alkaline phosphtase activity all through metamorphosis, a significant increased activity thereof was observed on day 5 in the pupating larva of *P. ricini* suggesting its possible role in the hormonal control during moulting. Likewise *A. mylitta* pupa also exhibited an increased activity on day 82 at mid-pupal stage.

Diapausing *A. mylitta* pupae when exposed to various photoperiodic regimens acid and alkaline phosphatase as well as Phosphorylase activity became highly predominant in LD groups 18:6, 14:10 and 10:14 h. Since light indirectly influences enzymic activities in insect tissues and direct exposure to it regulates the synthesis and release of their neurohormones (Danilevskii, 1965) the various metabolic processes also get stimulated. Thus insects exposed to longer light period especially the LD groups 18:6 h exhibited high enzymic activity while the reverse was true in the groups exposed to longer dark periods (LD 0:24).

The diverse behaviour of the two insects belonging to the same order Lepidoptera when exposed to different photophases during growth and development makes one wonder whether this diversity within the same order is also a biochemical characteristic of the class insecta.

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