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GLYCEROL VARIATION IN *PHILOSAMIA RICINI* HAEMOLYMPH DURING LARVAL-PUPAL DEVELOPMENT

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ABSTRACT

Studies on glycerol concentration in the haemolymph of *P. ricini* during larval-pupal development reveals that the initial low level during the fourth instar stage increases gradually with growth and the highest glycerol accumulation occurs in the fifth instar larva. On the onset of metamorphosis, after a significant depletion, once again glycerol accumulates during puparium-pupa transformation whereafter till ecdysis a U-shaped pattern is observed.

INTRODUCTION

GLYCEROL an important component of all glycerides that constitute the neutral fraction of lipids has been reported by Wyatt and Meyer[1] to occur in the free form in the pupal haemolymph of the silk moth *Hyalophora cecropia.* As the pupal development proceeds there is a gradual accumulation but when the diapause is broken and adult development commences, glycerol rapidly disappears. This finding of Wyatt and his co-workers[1-3] was extended to other species of insects and subsequently confirmed[4,5]. Independently Chino[6,7] reported the occurrence, not only of glycerol but also of sorbitol in *Bombyx mori* embryo during diapause. Curiously however, the presence of glycerol was not detected in the larval blood of all the insects investigated.

Detection of glycerol during paper partition chromatography of larval haemolymph of *Philosamia ricini,* the Eri silk worm, prompted the study of its variation in the haemolymph of this economically important insect during larval-pupal development.

MATERIALS AND METHODS

Larvae of *P. ricini* were reared in the laboratory as described earlier[8].

Haemolymph was collected and pooled in three lots, each from 12 chilled larvae picked at random from the colony during different developmental stages. A small incision was made on the dorsal side of the insect with a sharp stainless steel blade and the pale yellow coloured fluid was allowed to ooze in a centrifuge tube kept immersed in crushed ice and containing a few crystals of thiourea to prevent darkening of the haemolymph.

To obtain pupal haemolymph, an incision was made on the anterior end of the dorsal side of the pupa with a sharp pair of scissors and by applying gentle pressure, the fluid was collected in a pyrex container kept cooled in freezing mixture.

Presence of glycerol in the larval and pupal haemolymph was detected by paper partition chromatography employing a mixture of butanol-acetic acid-water (5 : 1 :2, v/v/v) as irrigating solvent. Glycerol was visualized on the dried chromatogram as described by Hough[9] and Evans and Dethier[10] by spraying it first with sodium periodate solution (0·5% w/v) followed by freshly prepared silver nitrate [5%, w/v, –ammonia (sp. gr. 0·8, 5 : 1, v/v)] mixture after drying the chromatogram. Appearance of dark spots having the same R*f* as that of the authentic reference spot of glycerol along with the test solution on the same chromatogram in three different solvent systems cosfirmed the presence of glycerol.

The chromatogram on being left overnight, developed another spot having a lower R*f* than the reference sorbitol. This compound is under investigation.

Glycerol was estimated by the method of Lambert and Neish[11] as modified by Korn[12]. A suitable volume of haemolymph was deproteinized by the addition of an equal volume of trichloroacetic acid (10%, w/v) and after allowing to stand in the cold for 2–3 min. was centrifuged at 3000 rpm for 10 min. Aliquots 0·5–1 ml) from the supernatant in triplicates were employed for glycerol assay.

RESULTS AND DISCUSSION

With an initial high concentration after ecdysis to the fourth larval instar stage (Fig. 1), glycerol appears to have been utilized as evinced by its significant depletion in the two-day old larva. This is subsequently, followed by a period of accumulation all through fourth instar stage and culminating maximally on the eve of pupation. However, immediately after pupation, glycerol content gets depleted in the zero day pupa where it stands at the lowest level ever recorded during larval development.

During metamorphosis glycerol accumulates at the time of the transformation of the puparium to pupa on days 3/4 whereafter till emergence, it traces more or less a U-shaped pattern with minimal concentration on days 9 and 10. On emergence even (day 15) however, glycerol once again records the same

concentration as during puparium-pupa transformation.

Despite the fact that glycerol is a normal metabolic product in animals, its occurrence in appreciably high concentrations in insect blood and tissues at various stages of development is rather extraordinary[4-6]. However, its accumulation was observed exclusively at the stage when the species underwent diapause during adverse conditions of environment in winter.

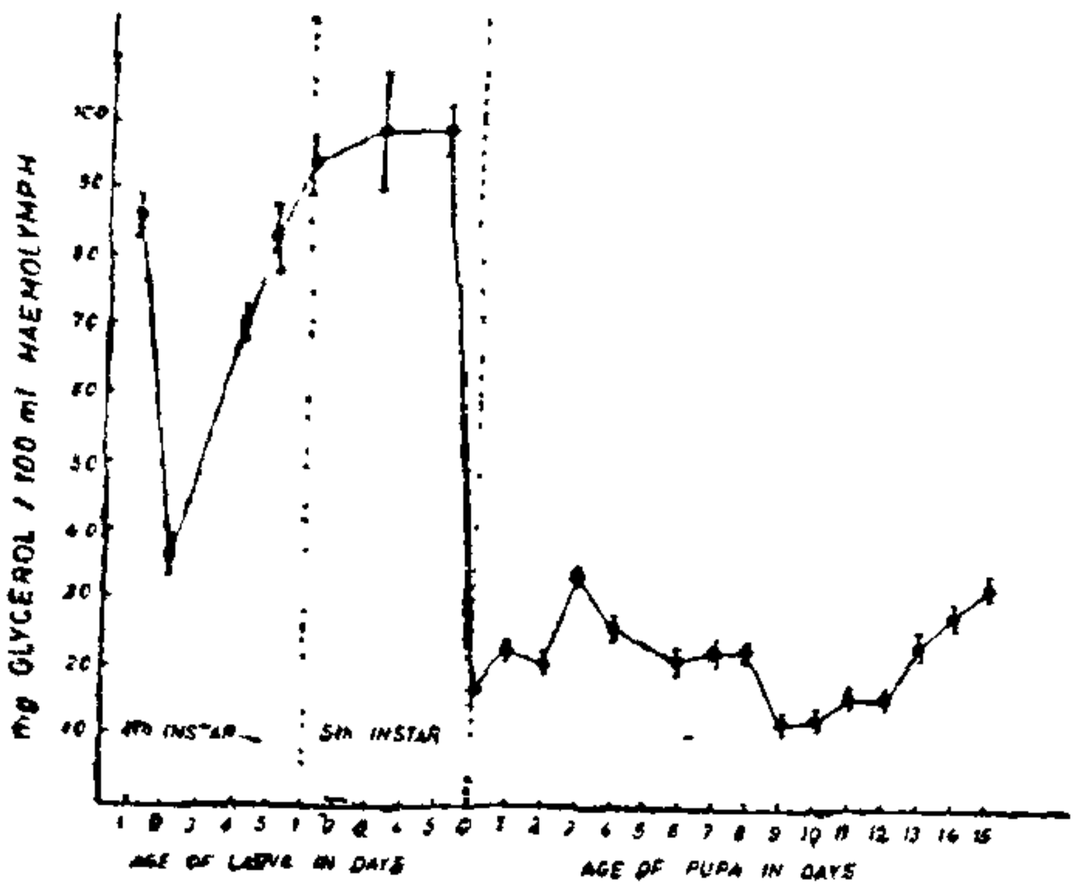

FIG. 1. Variation in haemolymph glycerol concentration during larval-pupal development of *P. ricini*.

Protection from tissue injury due to freezing by the addition of glycerol[13] led to the suggestion and subsequent confirmation[4-5] that glycerol accumulation in insects was a physiological adaptation providing resistance to low temperature. However, Wyatt and Meyer's[1] observations hardly support any such correlation between the two. Likewise, the present investigation in *P. ricini* also does not record any relationship between the presence of glycerol and cryoprotection and what is more *P. ricini* is a non-diapausing lepidopteran.

Following Chino's demonstration[7] of the appearance of glycerol with the simultaneous disappearance of glycogen in *B. mori* tissue, glycogen assays were carried out in the haemolymph of *P. ricini*. However, the results did not afford any supporting data to presume glycogen to be the source of glycerol.

Lepidopteran insects feed voraciously during larval development and consequently exhibit extremely high rate of metabolic activity. Therefore the accumulation of the highest concentration of glycerol during this period suggests high lipolytic and low α-glycerophosphate dehydrogenase activity both contributing to the formation and accumulation of glycerol.

Earlier investigations[14] have revealed that total lipids increase steadily through larval development and attain the maximum at the fifth instar stage However, on the commencement of spinning, it is followed by a steep fall and then by a rise whereafter an almost constant level is maintained. The variation pattern of lipolytic activity[15] during embryonic and larval-pupal development also further supports the view that the haemolymph glycerol has its origin from lipids.

The lipids that are synthesized in the alimentary tract from the ingested food by a phytophagous insect are transported *via* haemolymph to different tissues. Since neutral lipids are incapable of being transported as such, the intestinal lipase brings about their hydrolysis to mono, di and triglycerides along with the simultaneous release of free glycerol and free fatty acids in the haemolymph. On the other hand, the biosynthesis of neutral lipids from the available constituents occurs in the fat body of the insect. Accordingly, it could be speculated that the fat body withdraws glycerol from the haemolymph as and when required for esterification, suggesting that glycerol variation in larval haemolymph is indicative of lipolysis and lipogenesis occurring in the alimentary tract and the fat body.

Developing adult tissues of *H. cecropia* have been observed to incorporate labelled glycerol at a high rate[16]. Thus the role of glycerol in insects could be considered not only as a cryoprotective agent[17] but also as a precursor of phospholipids and triglycerides.

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EFFECT OF THE SCORPION, *HETEROMETRUS FULVIPES*, VENOM ON GLYCOLYTIC ACTIVITY IN THE BRAIN, MUSCLE AND LIVER OF FROG

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ABSTRACT

The effect of *Heterometrus fulvipes* venom on glycolytic activity was studied in frog brain, muscle and liver during a period of 48 hours. There was a rapid increase in total (*ab*) and active (*a*) phosphorylases in the three tissues both *in vivo* and *in vitro* conditions. The increase was more in liver than muscle and brain. Glycogen content decreased significantly in the three tissues. Lactate dehydrogenase activity also increased uniformly in all the three tissues both *in vivo* and *in vitro*. Lactic acid level increased in liver and decreased in muscle and brain. All these changes occurred progressively reaching maximum around 12–24 hours and then onwards tending towards normal level except in necrotic muscle. These changes suggest sympathetic stimulation by *H. fulvipes* venom and the prevalence of anaerobic condition after venom poisoning.

INTRODUCTION

IT was observed that *H. fulvipes* venom suppresses aerobic enzyme systems and enhances lytic enzyme systems both *in vivo* and *in vitro* conditions in brain, muscle and liver[1,2]. *H. fulvipes* venom was also known to alter metabolic activities[1]. It was reported that *Leiurus quinquestriatus* venom inhibited catalase activity in human erythrocytes[3], while *Buthus minax* venom was found to inhibit succinic dehydrogenase and acetylcholinesterase activity levels[4] in mouse tissues. It was reported that scorpion venom produces hyperglycemia[5,6]. It was also known that the effect of venom varies with the scorpion species and the target tissue[7]. Hence the present study reports the *in vivo* and *in vitro* effect of the venom of the less virulent type of the scorpion (chactoid scorpion), *H. fulvipes*, venom on the glycolytic activity in brain, muscle and liver of frog, *Rana hexadactyla*, which was found to be relatively resistant to venom action[1].

MATERIALS AND METHODS

Venom was collected from the scorpions by applying electrical stimulation to the postabdominal region of the animal and was collected into microbeakers kept at ice-cold temperature. Protein content of the venom, estimated by Lowry *et al.* method[8], was taken as criterion to express the venom quantity. Glycogen[9] and lactic acid[10] contents were estimated in normal and experimental tissues. Phosphorylase (*a* and *ab*) was assayed in the direction of glycogen synthesis[11]. The enzyme activity was expressed as μ moles of Pi formed/mg protein/hour. Lactate dehydrogenase activity was assayed using lithium lactate as substrate[12]. The enzyme activity was expressed as μ moles of formazan formed/mg protein/hour. *In vitro* effect of venom on enzyme activities was studied by the addition of 100 μg venom to tissue homogenate. *In vivo* studies were made during a period of 48 hours after sublethal doses of venom administration (intramuscular). One-third LD_{50} was taken as sublethal doses. LD_{50} was determined separately[13]. Statistical analysis of the data was done according to the method of Pillai and Sinha[14].

RESULTS AND DISCUSSION

Phosphorylase (*a* and *ab*) activity increased due to *H. fulvipes* venom both *in vitro* and *in vivo*. *In vitro* addition of 100 μg venom produced a rise in phosphorylase '*ab*' level to a greater extent than phosphorylase '*a*' in brain, liver and gastrocnemius muscle of frog (Table I). *In vivo* administration of sublethal doses of *H. fulvipes* venom produced a steep rise in phosphorylase (*a* and *ab*) activity in liver even by 6 hours after venom administration while the increase was not significant in brain and muscle at that time (Fig. 1). There was a lag in the increase of activity upto 6 hours in brain and then there was a sudden