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SOME STUDIES OF THE HÆMOLYMPH OF *LUCILIA CUPRINA* (CALLIPHORIDAE: DIPTERA)

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Abstract.- The haemolymph proteins, glucose and lipids of different developmental stages of *Lucilia cuprina* were estimated quantitatively. Starting with the early second instar, the protein contents decreased gradually as the fly reached the late pupal stage. The glucose level increased prior to the each larval molt but decreased as the pupa grew old. The lipid contents were higher in the early second instar larvae and decreased prior to molting, but in the third instar the values were reversed and in pupa also this value increased with age.

INTRODUCTION

Haemolymph which is the only extracellular fluid in insects exists in an unbound and non-vascular state circulating freely around the various body tissues in the haemocoel. The chemical composition, therefore, is very important in the general functioning of the body. The insect blood constituents are profoundly affected by different environmental stresses like temperature, insecticides, anaesthesia, and food (Dahlman, 1975; Jutsum *et al.*, 1975), so their study is important in order to understand the mechanism by which they change. These variation in haemolymph constituents as related to growth and metamorphosis are also important for a clear understanding. Some work has already been done on this aspects including that of Hill and Goldsworthy (1968); Green and Dahlman (1973); Firling (1977); Woodring *et al.* (1977) and many others.

The insect haemolymph is not concerned with respiration so it lacks a respiratory pigment apart from some chironomids, but it has various inorganic and organic constituents which play different roles in various insect phenomena. One of its chief functions is the transport of assimilated nutrients to sites of synthesis and storage and carrying the mobilized reserves to the tissues which require them. In this way the different constitutents, especially the organic ones, are in a constantly fluid state of concentration. This fluidity is very marked during metamorphosis as has also been reported by various previous workers like Wyatt (1961); Jeuniaux (1971); Florkin and Jeuniaux (1974); Woodering *et al.* (1977). Proteins, lipids and carbohydrates are important organic constituents and involved intimately in different developmental processes.

This changing concentration of the haemolymph proteins is an indication of the change in the synthetic activity associated with the differentiation processes taking place in the developing insect. Apart from taking part in the building up of body tissues an important portion of haemolymph proteins consists of a number of enzymes or isoenzymes (Laufer, 1960), e.g. proteolytic enzymes which are present in the pupa and tyrosinase which is especially important in dipteran puparium, as it is needed here for the hardening and darkening of the cuticle. Lipids are also transported via the haemolymph and wide variations in haemolymph lipid concentration occur during different processes like development, metamorphosis, flight and oogenesis (Florkin and Jeuniaux, 1974).

Haemolymph is also a likely site for carbohydrate metabolism as the presence of several concerned enzymes has been demonstrated in the blood of several insects. The presence of glucose as one of the reducing substances has also been reported and discussed by several authors including Wyatt (1967) and Florkin and Jeuniaux (1974).

Considering the importance of these substances their concentration pattern was studied in the haemolymph of the different developmental stages of *Lucilia cuprina*.

MATERIALS AND METHODS

Collection and maintenance of the flies

Lucilia cuprina used during the present work were taken from a colony maintained in the laboratory. They were reared at 28±1°C, 12 hours photoperiod and retative humidity ranging from 65% to 70%. These flies were given *ad libitum* access to both protein and sugar source in the form of fresh minced beef and pieces of peeled banana.

The estimation of haemolymph proteins, glucose and lipids was done for the early and late (just after and prior to the next molt) second and third instar larvae and pupae of different ages (1, 48 and 96 hours old). For the late third instar larvae, immobile larvae, (prior to puparium formation) were used. The first instar larva was found too small to yield sufficient blood for various biochemical tests. To determine the total protein contents of the haemolymph, method of Lowry *et al.* (1951) was employed. The quantitative analysis of the haemolymph glucose content was done by O-toluidine method of Hartel *et al.* (1969), while, lipid contents were determined by the method of Zollnar and Kirsch (1961).

RESULTS

Haemolymph protein contents

The total haemolymph protein level of the early second instar larva was found to be 13.83 g/100 ml of the haemolymph. In the late larval stage this level of the haemolymph proteins had dropped to 9.25 g/100 ml of the blood. In the early third instar larvae it was 9.05 g/100 ml of the blood, which had a lower value than the protein level of the corresponding stage of the second instar. In the late third instar this level further dropped down to 7.70 g/100 ml of the haemolymph.

The haemolymph protein contents decreased gradually as the pupa grew old. This level was 6.66 g/100 ml of the blood after 1 hour of the onset of the pupal life. In 48-hour pupa it was 4.96 g/100 ml while in 96-hour pupa it had dropped to 2.30 g/100 ml of the haemolymph. The amount of proteins present per 100 ml of the haemolymph of different developmental stages is shwon in Table I.

TABLE I.- HAEMOLYMPH PROTEINS OF DIFFERENT DEVELOPMENTAL STAGES OF *LUCILIA CUPRINA*.

Age		Amount of protein (mg/100 ml of blood)	± S.E.M.
Second instar larvae			
	Early	13.83	± 0.0833
	Late	9.25	± 0.4430
Third instar larvae			
	Early	9.05	± 0.2783
	Late	7.70	± 0.5195
Pupae			
	1 hour	6.66	± 0.1666
	48 hour	4.96	± 0.2603
	96 hour	2.30	± 0.152

Haemolymph glucose

The haemolymph glucose level of the early second instar larva was 71.79 mg/100 ml of the blood. This level increased to 85.71 mg/100 ml of blood in the

late second instar larval stage. In the early third instar larva it was lower than the late third instar larval stage. It was 82.36 mg/100 ml in the former but 88.88 mg/100 ml of the haemolymph in the latter.

Like the protein values the glucose level also decreased gradually with age in the pupa. This amount was 72.22 mg/100 ml, 66.66 mg/100 ml and 38.88 mg/100 ml of the blood in the 1 hour, 48-hour and 96-hour old pupae respectively. The haemolymph glucose level of the different larval and pupal stages is shown in Table II.

TABLE II.- HAEMOLYMPH GLUCOSE OF DIFFERENT DEVELOPMENTAL STAGES OF *LUCILIA CUPRINA*.

Age		Amount of glucose (mg/100 ml of blood)	± S.E.M.
Second instar larvae			
	Early	71.79	± 6.7819
	Late	85.71	± 8.2474
Third instar larvae			
	Early	82.36	± 8.9851
	Late	88.88	± 11.1133
Pupae			
	1 hour	72.22	± 20.0316
	48 hour	66.66	± 0.00
	96 hour	38.88	± 5.555

Haemolymph lipids

The total haemolymph lipid level of the early second instar larva was 131.98 mg/100 ml of the blood, but it had dropped down to 90.37 mg/100 ml in the late instar. In the early third instar it was 96.35 mg/100 ml but in the late third instar it had increased to 99.77 mg/100 ml of the haemolymph.

In pupa also the lipid level increased gradually with age. The total haemolymph level was 105.66 mg/100 ml of the blood after one hour of the onset of the pupal life, but it had increased to 112.18 mg/100 ml in the 48 hour pupa while in 96 hour pupa it was 123.33 mg/100 ml of the blood. The total haemolymph lipid level of the different larval and pupal stages is shown in Table III.

TABLE III.- HAEMOLYMPH LIPIDS OF DIFFERENT DEVELOPMENTAL STAGES OF *LUCILIA CUPRINA*.

Age	Amount of lipids (g/100 ml of blood)	± S.E.M.
Second instar larvae		
Early	131.98	± 13.73
Late	90.37	± 8.99
Third instar larvae		
Early	96.35	± 6.93
Late	99.77	± 7.79
Pupae		
1 hour	105.61	± 6.976
48 hour	112.18	± 9.145
96 hour	123.33	± 1.089

DISCUSSION

The pattern of changes in the concentration for the haemolymph constituents which have been studied here is different in the case of lipids and proteins but resembles in the case of proteins and glucose. Although it may be due to the regulation of all the blood constituents during each developmental instar as a result of feedback induced by the changing concentration, but Woodring *et al.* (1977) considers feeding and growth during the molting cycle equally important. Green and Dahlman (1973) also considered feeding an important influence which governs the pattern of concentration of different blood constitutens during metamorphosis.

Haemolymph protein concentration

The amount of protein was 13.83 g/100 ml of haemolymph in the early second instar of *L. cuprina* which was the highest concentration detected in this insect during metamorphosis. After this there was a decrease in the concentration per unit of the haemolymph. With the onset of the pupal life this concentration came down to nearly half of that found in the early second instar and towards the end of the pupal life the amount of haemolymph proteins was about one third of the concentration found in early pupal blood. These results show a different pattern of concentration as compared to what is found in some other insects,

while comparable conditions are present in others. In *Bombyx*, a rise in haemolymph proteins has been reported from the early third instar to the late fifth instar larvae (Florkin, 1937; Wyatt *et al.*, 1956). A similar increase has also been noted in *Galleria mellonella* (Denuce, 1958), *Samia cynthia* (Laufer, 1960), *Pieris brassicae* (Chippendale and Kilby, 1969), *Diatraea grandiosella* (chippendale, 1970) and other Lepidoptera. An increase in protein concentration has been reported for some other insects like *Popillia japonica* (Ludwig, 1954), *Drosophila* and *Culex* (Chen, 1956) and *Pyrrhocoris apterus* (Emmerich, 1970).

But like the present study many workers have reported a decrease in the haemolymph protein concentration towards ecdysis or during apolysis, that is prior to molting. For example Hill and Goldsworthy (1968) and Woodring *et al.* (1977) noted a sharp decrease in the blood protein concentration in locusts and *Acheta* respectively. Firling (1977) has also reported a decline towards the formation of pharate pupa in *Chironomus*, although he noted the increase in the haemolymph protein concentration from the early to the late fourth instar larva of this fly. This pattern is not consistent in all the insects going through metamorphosis as has been shown by Green and Dahlman (1973) while working on the larvae of *Manduca sexta*. In these larvae the change in pattern was consistent throughout the larval stages except the fifth instar. The early instars showed a decrease in the early and late stages which they called phase-I and phase-V respectively, while during the rest of the life the larval instar showed an increase. In the fifty instar, on the other hand, there was a decrease even in mid-life or phase-III.

In *Lucilia* also feeding might play an important role in the establishment of the changing pattern of haemolymph proteins. This change in pattern can be due to the appearance and disappearance of some proteins or to a relative increase and decrease in its components as has also been found by other workers (Chen, 1966; Martin *et al.*, 1969, 1971; Green and Dahlman, 1973; Firling, 1977). The consistent decrease in the haemolymph protein concentration of *L. cuprina* during metamorphosis can be explained by their continuous synthesis and then rapid utilization for growth, or due to a reduced synthesis and increased utilization or increased retention by the fat body. The uptake of larval hemolymph proteins by the salivary glands and other tissues of the body like gut, as suggested for *Pieris* (Chippendale and Kilby, 1969), *Diatraea* (Chippendale, 1970) and *Chironomus* (Firling, 1977) could be responsible for the depletion of these proteins. In the pupa of *L. cuprina* there was a marked decrease in the concentration of haemolymph proteins. During this stage extensive histolysis and histogenesis takes place. The decomposition of protein contents to peptides or amino acids apart from the above mentioned reasons could collectively lead to a decreased concentration. The utilization of tyrosinase for the hardening and

darkening of the cuticle of the aging puparium has been discussed by Chen (1966) and this like other haemolymph enzymes leads to changes in the haemolymph protein concentration. These ontogenetic variations in the haemolymph protein contents are the net results of synthesis, utilization, transport, storage and decomposition as proposed by Firling (1977) for *Chironomus* larvae.

Haemolymph glucose concentration

The presence of glucose as a fermantable substance in the insect haemolymph is quite common. In *L. cuprina* in the second and third larval instars the glucose level rises as these instars grow old. Although the increase is more marked in the former than the later instar but the initial concentration is higher in the third instar. In 1-hour pupa this level is comparable to the early second instar but it decreases as the pupa grows old until in 96-hour pupa there is about 50 percent decrease. Although the main blood sugar is trehalose, it is present in the adult honey bee (Czarnovsky, von 1954), and in *Phormia regina* where trehalose appears at pupation but is absent in larval instars (Evans and Dethier, 1957). In the cockroach *periplaneta americana* it is present, although in very low quantities at any given time (Methews *et al*., 1976). According to Wyatt (1967) glucose is important in carbohydrate metabolism as trehalose can be syntehsized directly through the intermediates glucose-6-phosphate and trehalose-6-phosphate. Recent studies on the american cockroach, *periplaneta americana* have also shown that it can synthesize trehalose directly (Mathews and Downer, 1974; Spring *et al*., 1977). With the increase in data concerning the presence of glucose and other reducing mono-or disaccharides in the haemolymph, Florkian and Jeuniaux (1974) suggested that these sugars are a features of the haemolymph of Hymenoptera and Diptera well adapted to sustained flight.

Haemolymph lipid concentration

Wide variations in haemolymph lipid concentration are knwon to be a common phenomenon during metamorphosis (Florkin and Jeuniaxu, 1974). In *L. cuprina* haemolymph lipid concentration was highest in the early second instar larva but it had decreased considerably towards the end of this instar. In the third instar there was almost no change and the level remained more or less the same but with the start of the pupal life, a steady increase took place but even then this level did not exceed the amount present in the early second instar larva. In *Acheta* a doubling of concentration followed by a steady increase throughout the instar has been reported even though the food consumption essentially stops two or three days before apolysis. During the present study the same pattern of increase was observed except for the second instar larval where a considerable lowering of the concentration was noted. The lipid content of the haemolymph was

considerably more throughout the life of this blowfly as compared to that of glucose. In the larva the source is the gut from which it is transported via haemolymph to the fat body for storage and its subsequent release and passage to various organs. In the pupa its amount increased irrespective of the fact that no feeding takes place and here the reason for this rise in level could be dual, that is, those released from the fat body and those which get accumulated in the blood as a result of tissue break up.

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