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ROLE OF CORPORA-ALLATA AND BRAIN ON THE CARBOHYDRATE LEVEL OF HAEMOLYMPH IN *LOHITA GRANDIS* GRAY (PYRRHOCORIDAE: HETEROPTERA: HEMIPTERA)

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THE insect haemolymph, in addition to transporting nutrients and waste products between tissues can have a significant storage function, substances getting accumulated or utilised depending on the circumstances. But very little information is available on the distribution of solutes between the plasma and the tissues, although it is clear that the distribution can be very uneven and can change quickly. Such changes are of prime importance in regulating the process of metabolism. Further, the hormones from different sources (*e.g.* brain, corpora-allata, corpora-cardiaca) are also transported by the haemolymph and these play a major role in its biochemistry¹. L'Helias² first pointed out that in *Carausius* (a wingless stick insect), allatectomy brought about a steep decline in the level of blood sugar. According to Ralph and McCarthy³, the injection of saline extract of brain caused a rapid increase in the level of trehalose of haemolymph in *Periplaneta americana*. Goldsworthy⁴, in *Locusta*, noted that removal of cerebral neurosecretory cells resulted in a marked accumulation of carbohydrates in the haemolymph and commented that it was due to the metabolic changes brought about in the absence of cerebral neurosecretion. Gade⁵ observed species specificity, to the role of neurosecretion and hormones of corpus allatum/corpus cardiacum, on the metabolism of carbohydrates and observed that the neurosecretory corpora cardiaca and corpora allata complex of *Carausius* contained both adipokinetic and hyperglycemic factors that were responsible for the increased levels of lipids and carbohydrates in *Locusta* and *Periplaneta* respectively. The present investigation was undertaken to determine the level of carbohydrate

in the haemolymph of *Lohita grandis* after removal of brain and corpora-allata from the insect. Effect of application of Juvenoid (JHa) on carbohydrate level in allatectomized insects was also studied.

Adults were collected from the colonies reared in the laboratory by the method of Mandal *et al*⁶. The corpora-allata was removed by the methods of Stay and Tobe⁷ and brain-cauterization performed by the technique of Girardie⁸. In all cases during operation, the insect's Ringer solution used was mixed with a few crystals of phenyl thiourea and the operated area was immediately sealed with the sterile "Bees-wax" impregnated with phenyl-thiourea. The juvenile hormone analogue (JHa) used was N-(2,5-dichlorophenyl)-3,7-dimethyl-2,6-octadienylamine obtained from Prof. A. M. De Oliveira Filho (Brazil) and was injected into the allatectomized insects at a dose of 20 µg in 10 µl acetone by a tuberculin microsyringe (Hamilton, USA). The control insects received only 10 µl of acetone/insect. For each treatment the experimental insects were sacrificed at 24, 48 and 72 hr after treatment. The haemolymph was collected by the graduated glass capillary tube and stored at 0°C for biochemical analysis. The total carbohydrate level of haemolymph was estimated according to Mordue and Goldsworthy⁹ while the trehalose was estimated according to Roe¹⁰. Glucose was estimated by the glucose oxidase method¹¹. The volume of haemolymph was determined by the dye dilution technique¹². The contents of carbohydrates were expressed per mg haemolymph protein; and the total protein content was determined by the methods of Lowry *et al*¹³. The experimental data were analysed statistically by the students-*t*-test and Duncan's multiple range test.

It was found that allatectomy resulted in a sharp decline of the total carbohydrate, trehalose, and glucose level in the haemolymph as compared with the sham-operated control cases (table 1). The post-treatment exposure also exerted a significant effect *i.e.* the decline was more pronounced in the 72 hr than either in 24 hr or in 48 hr after operation (table 1). The volume of haemolymph increased significantly ($P < 0.01$) after the removal of corpora allata (table 2). Application of JHa into the allatectomized insects reversed the effects of allatectomy *i.e.* it increased total carbohydrate, trehalose and glucose levels and a drop in the volume of haemolymph compared with the allatectomized insects (tables 1 and 2) was noticed. Compared with other treatments, the removal of brain resulted in a significant increase ($P < 0.01$) in the total carbohydrate and glucose contents and fall in tre-

Table 1 Changes in the concentrations of total carbohydrate, trehalose and glucose in the haemolymph of female *Lohita grandis* before and after surgical operations and juvenoid treatment.

Treatment	Total carbohydrates ($\mu\text{g}/\text{mg}$ protein)	Trehalose ($\mu\text{g}/\text{mg}$ protein)	Glucose ($\mu\text{g}/\text{mg}$ protein)
Control**	9.3 (0.3)	5.3 (0.6)	1.3 (0.2)
Allatectomy			
24 hr.	7.4 (0.2)	5.0 (2.4)	0.9 (0.9)
48 "	7.0* (0.2)	4.2* (0.4)	0.6* (0.31)
72 "	7.2* (0.9)	3.6* (1.0)	0.5* (0.1)
Allatectomized + Juvenoid ⁽¹⁾ treated			
24 hr.	9.9 (1.4)	6.2 (2.3)	1.0 (0.1)
48 "	10.6* (0.8)	7.0* (0.5)	1.3* (0.1)
72 "	12.6* (0.3)	8.0* (0.3)	1.3 (0.22)
Brain-cauterization			
24 hr.	17.0* (0.5)	5.1 (1.2)	2.1* (0.5)
48 "	16.4* (0.1)	2.3 (0.7)	2.9* (0.2)
72 "	16.1* (0.5)	3.1* (0.9)	2.5* (0.2)
Allatectomized + Brain-cauterized			
24 hr.	6.3* (0.3)	4.1* (0.2)	0.9* (0.1)
48 "	6.1* (0.6)	4.3* (0.9)	0.8* (0.1)
72 "	5.2* (0.6)	3.6* (0.7)	0.4* (0.1)

(1) Here the juvenoid was the N-(2,5-dichlorophenyl)-3,7-dimethyl-2,6-octadienylamine.

** As there was no difference between the controls of each treatment only the mean values are given here. All other data are the mean \pm S.E. (within the parentheses) where $n = 15$; * indicates the significant differences in comparison to that of control ($P < 0.01$).

Table 2 Changes in haemolymph volume in female *Lohita grandis* Gray before and after surgical operations and juvenoid treatment.

Treatment (hr)	Haemolymph volume	
	($\mu\text{l}/\text{insect}$) Mean	\pm S. E. M. ($n = 15$)
Control**	180	6.3
Allatectomy		
24	192	4.0
48	205*	6.3
72	228*	3.0
Allatectomized + Juvenoid ⁽¹⁾ Treated		
24	172	10.2
48	153*	4.2
72	142*	5.1
Brain-cauterization		
24	185	5.0
48	188	2.6
72	190*	1.3
Allatectomized + Brain-cauterized		
24	155*	8.2
48	150*	12.2
72	129*	8.0

(1) Here the juvenoid used was the, N-(2,5-dichlorophenyl)-3,7-dimethyl-2,6-octadienylamine.

** As there was no significant difference between the controls of each treatment, only the mean values are given here. Other details are as in table 1.

halose level of haemolymph (table 1). The volume of haemolymph also increased after brain-cauterization (table 2) as obtained in the case of allatectomy but in this case, the effects were significant ($P < 0.01$) only 72 hr after operation. Simultaneous removal of both corpora-allata and brain showed that all fractions of carbohydrates estimated decreased more significantly ($P < 0.01$) than any other treatment (table 1). The most interesting result obtained in this case was that the volume of haemolymph decreased significantly ($P < 0.01$) (table 2) whereas after allatectomy and brain-cauterization separately, it increased significantly (table 2).

In insects, the neuro-endocrine system is known to regulate certain aspects of metabolism such as oxygen uptake, synthesis and breakdown of trehalose, lipids and proteins¹⁴ which in turn regulate the concentrations of metabolites and enzymes in the haemolymph. The reduction in the level of carbohydrates (trehalose, glucose and total carbohydrate) in the haemolymph after removal of corpora-allata and increase in the volume of haemolymph as seen here corroborated the findings of other earlier workers^{2, 15-17}. The treatment of JHa in the allatectomized insects again induced a rapid increase in the contents of all the carbohydrates in the haemolymph which decreased after CA removal and this sort of fluctuation in the level of different carbohydrates in the haemolymph after the removal of CA and JHa treatment was probably due to the differential rate of oxygen consumption in the body of insects and also to the induction and inhibition of different enzymes system by CA hormone^{14, 18-19}. According to earlier work^{2, 17} the CA performed the diabetogenic function in adult insects. The present experimental results also confirmed the diabetogenic nature of CA in insects. The increased storage of total carbohydrates and glucose in the haemolymph of *Locusta* after brain cauterization agreed with similar findings in other insects^{1, 4} and it was probably due to the absence in the haemolymph of carbohydrate mobilising factor(s) produced by the cerebral neurosecretory cells^{4, 20}. Similarly the increased volume of haemolymph after brain cauterization as noticed here also corroborated the previous findings⁴. The significant drop in the level of trehalose in the haemolymph after brain-cauterization was probably due to the induction of trehalase enzyme as already reported by Mandal and Choudhuri²¹. But why the removal of both CA and brain, from the insects at a time, produced the different results in respect of the contents of carbohydrate, in the haemolymph and caused a change in the volume of

haemolymph was not clear. This might be due to the imbalance caused in the physiology by the removal of two important neuro-endocrine centers which ultimately induced the senescence process directly or indirectly into the insect²².

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