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Changes of Digestive Enzymes during the Post-embryonic Development of *Lohita grandis* Gray. I. Effect of Allatectomy, Brain-cauterization and Juvenoid Treatment

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This paper presents the quantitative changes of five major digestive enzymes during the post-embryonic development in *Lohita grandis* a heteropteran insect treated in different ways. It incorporates the effects of allatectomy, decapitation and juvenoid treatment on the secretion and synthesis of the enzymes in adult insects. The enzymes studied are the total protease, amylase, lipase, trypsin and chymotrypsin. During the early phage of development the total protease, amylase, and trypsin activity are higher whereas the chymotrypsin and lipase activity are relatively very insignificant. Chymotrypsin activity reaches its maxima in fifth nymphal stage whereas lipase shows its maximum activity during the fourth nymphal stage to adult. The total protease, amylase and lipase activities are found in fore gut, mid gut and also in hind-gut with mid-gut showing the highest activity. Trypsin and chymotrypsin activity are found mostly in the mid-gut, with fore-gut showing only traces or no activity. Allatectomy in adult insects leads to the significant decrease of total protease, lipase, trypsin and chymotrypsin activity but amylase shows no significant differences. Brain cauterization also shows the same effect as in case of allatectomy but in this case amylase activity also decreases. In case of allatectomy coupled with brain cauterization done in an insect drop in the activity of all enzymes to a large extent is found. Treatment of juvenoid in allatectomized insects reverses the effect of allatectomy and when juvenoid applied to the allatectomized and brain cauterized insect it also shows the same effect but the effect of juvenoid is more pronounced in case of allatectomized insect. The physiological significance of this effect has been discussed.

Key Words: *Lohita grandis;* **Digestion; Enzymes; Allatectomy; Brain-Cauterization; Post-embryonic development; Juvenoid**

Introduction

Very little information is so far available regarding the nature, qualitative and quantitative variation of digestive enzyme during the post-embryonic development of insects (Mandal et al. 1981 a, b). This is mainly due to the difficulties in collection of enzymes from the gut during early phase of development and also for the lack of adequate differentiation between secreted enzymes (Terra et al. 1979). Mandal et al. (1981 a) observed a significant variation in quantitative and qualitative activity of digestive enzyme during post-embryonic development of *Schizodactylus monstrosus*. It has been established that insects which live on food rich in some particular substance generally produce the relevant enzymes in appropriate volume (Cheung & Gooding 1970, Wigglesworth 1972 and House 1974), and the activity and concentration of these enzymes in the gut are supposed to be controlled by several factors like supply of food (Englemann 1968, 1969), secretions of neuro-secretory cells (Thomsen & Moller 1963), and frontal ganglian (Clarke & Gillott 1967) and the secretion of corpora allata (Wigglesworth 1936, 1948, Dadd 1961 and Mandal et al. 1981 a, b). The increase in the activity of digestive enzymes in *Schizodactylus monstrosus* after topical application of juvenoid as reported by Mandal et al. (1981 a) was probably the first indication of juvenoid influence on enzyme activity.

In this communication attempts have been made to study the qualitative and quantitative changes in the activity of five major digestive enzymes in the gut, lumen and mid gut cells in *Lohita grandis* during post-embryonic development. In addition the study has also been extended to analyse effects of allatectomy brain-cauterization and juvenoid treatment on the synthesis, distribution and activities of enzymes in various regions of the alimentary canal in adult insect.

Material and Methods

Materials

The substrates used bovine serum albumin (fraction V), soluble starch and peptides (all of BDH England) and pure olive oil (Bertolli, Italy). The other reagents used were either of E. Merck (Darmstadt, Germany), BDH (India) or Sigma Chemical Company (USA) and were of analytical grade. The solutions were prepared in double glass distilled water.

Animals

Specimen of *Lohita grandis* (Heteroptera: Pyrrhocoridae) were reared in laboratory under 16 h light; 8 h dark regime at 25°C, 75% RH and were provided with a diet containing 10% sucrose solution and extracts of leaves on which the insects generally feed.

Hormone treatment

The juvenile hormone analogue used was the compound FMC 23509 (CRD 9499) being dissolved in double distilled acetone and it was applied topically with food in a dose of 25μg/10μl/insect. The control insects received only the equivalent quantities of acetone.

Allatectomy and Brain-cauterization

Corpora allata were removed from insects, just after the attainment of the adult form by following the technique of Stay and Tobe (1977) and the cauterization of brain was by following the technique of Girardie (1966) and operated area was sealed with melted wax substituted with phenyl thiourea. The operation

was done under freshly prepared insect ringer solution containing phenyl thiourea. (The mortality rate after operation was very high, about 50%). The insects were used for experimental studies 24 hrs. after operation.

Preparation of homogenates

The experimental insects were killed by submerging them in water and their alimentary canals were dissected under the chilled saline and taken out leaving aside adhering fat-bodies, malpighian tubules, mycetomes and other materials. Each gut was then divided into three parts Fore, Mid and Hind gut. From midgut the luminal fluid was washed into a known volume of glass distilled water of neutral *p*H. Each part of the gut were then homogenized separately in a glass homogenizer containing glass distilled water of neutral *p*H. The homogenate of each gut fraction was then passed through a piece of nylon mesh (about 90 μm pore size) and through glass wool, and centrifuged at 8000g for 10 min under cold condition.

Assay for Enzyme activity

Amylase (after Bernfeld 1955)

Reaction mixture of 1 ml 0.5% starch solution, 2 ml phosphate buffer of *p*H 8 and 0.2 ml homogenate were incubated for 10 min at 37°C. The reaction was terminated by 2 ml of Dinotro-salicylic acid reagent and absorbance read at 500 nm.

Protease (after Snell & Snell 1971)

Reaction mixture of 1 ml 50 ppm BSA, 2 ml phosphate buffer of *p*H 11.2, 0.2 ml homogenate and one drop of 0.1 M $MgSO_4$ solution was incubated at 37°C for one hour. The reaction was terminated by 1 ml 50% TCA and the absorbance was measured at 660 nm.

Lipase (after Cherry & Crandall 1932)

Reaction mixture of 1 ml olive oil suspension (0.66%) 0.5 ml phosphate buffer of *p*H 8 and 0.2 ml homogenate was incubated at 37°C for 24 hour. The reaction was terminated by 3 ml of 95% ethanol and then was titrated with 0.05 N NaOH solution by microburrete (Hamilton) adding 2 drops of 2% phenolophthalein indicator against blank.

Trypsin (after Erlanger et al. 1961)

Reaction mixtures of 2 ml 0.2 M glycine/sodium hydroxide buffer of *p*H 9.2, 0.2 ml homogenate and 0.2 ml 36 mM substrate (DL benzoyl arginine p-nitroanilide dissolved in dimethyl formadide) was incubated at 37°C for 10 min. The reaction was terminated by the addition of 1 ml 0.25 M bicarbonate +carbonate+1% sodium dodecyl sulfate and absorbance was read at 405 nm.

Chymotrypsin: (after Webster & Prado 1970)

Reaction mixtures of 2 ml 0.2 M glycine/sodium hydroxide buffer *p*H of 9.2, 0.2 ml homogenate, 0.2 ml 5 mM substrate (N-benzoyl-L-Tyrosine ethyl ester) were incubated at 37°C for 10 min. The reaction was terminated by the addition of 1 ml 0.25M bicarbonate+carbonate+1% sodium dodceyl sulfate and the absorbance read at 420 nm.

The *p*H values and substrate concentration used in the assay procedures were selected on the basis of the earlier studies by Terra et al. (1979) and Mandal et al. (1981).

Statistical analysis

Analysis of variance and students T-test were performed for obtaining the critical difference.

Results

It has been established that in insects midgut and midgut caecae are the main sites of the synthesis of digestive enzymes accordingly the enzyme activity in other gut fractions was treated as luminal activity (Wigglesworth 1972).

Enzyme activity in the four gut fractions during the post-embryonic developmental stages

Activity expresscd in terms of enzyme unit/gut. region/min

During the early phase of development the total protease, amylase and trypsin activity were higher whereas the activity of chymotrypsin and lipase were relatively low (figures 1–5). Chymotrypsin reaches its maximum activity during the fifth nymphal stage while the lipase had its maximum activity during the fourth nymphal stage to adult (figures 2 & 5). The protease, amylase and lipase were active in fore-gut, mid-gut and also in hind-gut, the activity being maximum in mid-gut. In respect to these enzymes the luminal activity was much greater than that of the mid-gut tissue ($P < 0.05$). In most cases both trypsin and chymotrypsin were active in the mid-gut tissue and lumen while in the

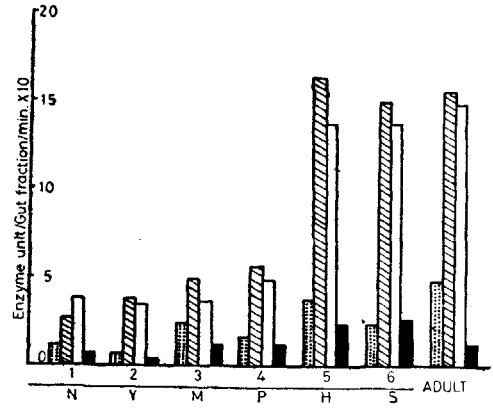

Figure 1

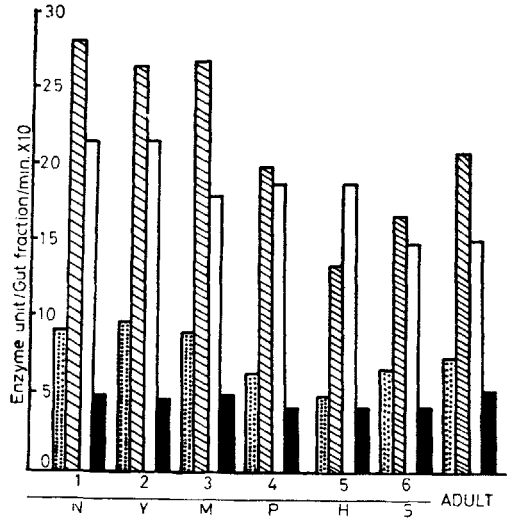

Figure 2

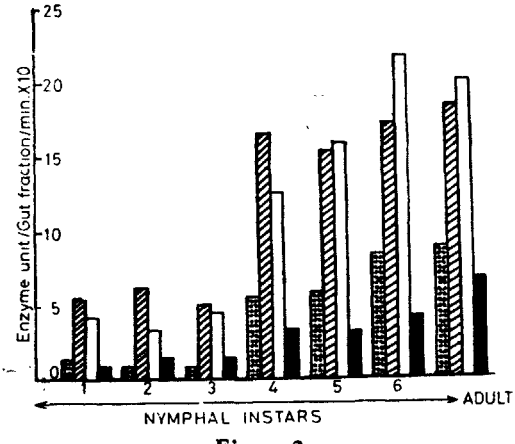

Figure 3

Figure 1 Changes of trypcin activity in four regions of the gut during the developmental period; 1-6 represent the respective nymphal instar; *Ad* represent adult form; dotted bar indicates the fore-gut fraction (FG); stripped bar indicates the mid-gut contents (MGC); blank bar is the mid-gut tissue (MGT) and the solid bar is the hind gut (HG) (Results are the mean of at least twentyseven determination in each case)

Figure 2 Changes of lipase activity in four regions of the gut during the developmental period (Others are same as in figure 1)

Figure 3 Changes of protease activity in four gut fractions during the post-embryonic developmental period (Others are same as in figure 1)

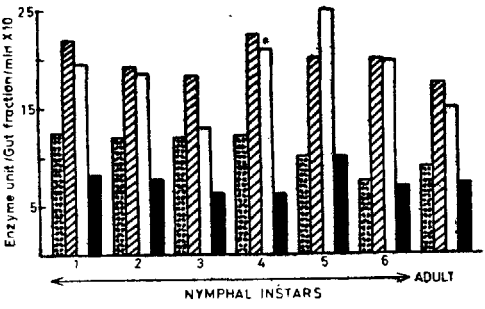

Figure 4 Changes of amylase activity in four gut fractions during the post-embryonic developmental stages (Others are same as in figure 1)

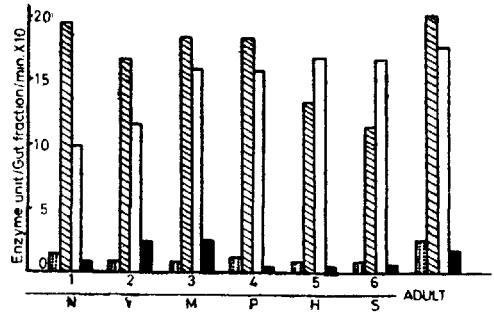

Figure 5 Changes of chymotrypsin activity in four-gut fractions during the post-embryonic developmental stages (Others are same as in figure 1)

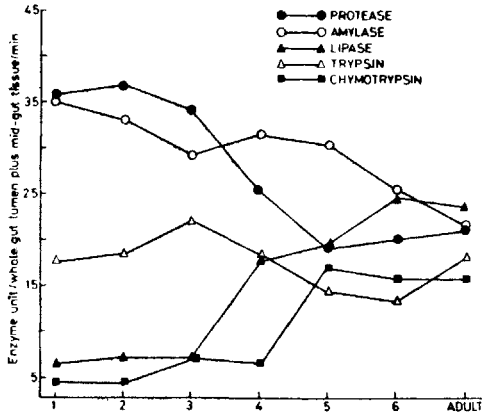

Figure 6 Relative fluctuation of protease, amylase, lipase, trypsin and chymotrypsin in relation to each others during the developmental period. *Activity expressed as a percentage of the total enzyme activity/whole gut lumen plus mid-gut tissue/min* (Others are same as in figure 1)

fore-gut and hind-gut the activity was either very low or altogether absent (figures 1 & 5). In the mid-gut tissue (MGT) and mid-gut contents (MGC) considerable variation in activity was found with almost all the enzymes. On comparison the activity of trypsin and chymotrypsin in fore and hind gut was always found to be lower than that of protease, lipase and amylase which suggest the probable presence of an inhibitor or inactivator in the fore and hind gut similar to that reported by Engelmann (1969) and Rao and Fisk (1965).

Results expressed as percentage of the total estimated enzymes/whole gut lumen plus mid-gut tissue/min.

The five estimated enzymes as already described showed the same relative fluctuation of their activity during the development (figure 6). In the first instar in comparison to the other developmental stages, protease and amylase showed the greatest activity with overall mean values of 35.79% and 35.11% whereas lipase had its maximum activity in the adult form with the mean value of 23.44%. Trypsin showed its peak activity in third nymphal stage and chymotrypsin in the fifth instar with the mean values of 22.04% and 16.82% respectively.

Total activity of the five enzymes in the gut lumen

The activity of each enzyme from the three regions of the gut lumen were summed to give the total luminal activity of each enzyme and the total activity of each of the five enzymes (estimated here) when summed gave the total enzymes activity of the whole gut lumen. Considering the total activity this way it has been found that the enzyme activity was at its highest level in the adult and then

to fifth instar stage where the values are 22.91 and 21.35 enzyme unit/whole gut lumen/min respectively. The lowest enzyme activity was at the third nymphal stage with 16.97 enzyme unit/whole gut lumen/min.

Effect of brain-cauterization, allatectomy and juvenoid treatment on the enzyme activity in the four gut fractions of adult insects

Activity expressed as enzyme unit/gut region/min.

Allatectomy produced more or less similar changes to all the enzymes excepting amylase (table 1) and the changes appeared more marked in trypsin and chymotrypsin. After operation there was a significant decrease in the activity of protease, lipase, trypsin and chymotrypsin but the decline in amylase activity was not statistically significant. ($P < 0.05$). All the four gut fractions exhibited the similar results but enzymes from the mid-gut tissue (MGT) were more affected viz. the trypsin and chymotrypsin activity from MGC of operated insects significantly greater than that of MGT ($P < 0.01$). Similarly the cauterization of the brain in allatectomized insect leads to further decline in the activity of enzymes in all the gut fractions (table 2). The only difference being that in allatectomized as well as cauterised insects there was more drastic decline in the activity of all enzymes including amylase in comparision to that only allatectomized individuals. But the declining trend in the activity of trypsin and chymotrypsin in

Table 1 *Effect of allatectomy and juvenoids (JHa) on the distribution and activities of five digestive enzymes in the four gut regions. Activity expressed as enzyme unit/gut region/min*

Treatment	Fore-gut (FG)			Mid-gut tissue (MGT)			Mid-gut lumen content (MGC)			Hind-gut (HG)			Enzyme[1]
	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	
	0.74	5	0.05	1.50	5	0.16	2.06	5	0.42	0.50	5	0.02	P
	0.90	8	0.14	1.50	8	0.38	1.75	8	0.09	0.73	8	0.12	A
Control	0.88	5	0.22	2.00	5	0.52	1.83	5	0.33	0.66	5	0.07	L
	0.25	5	0.01	1.75	5	0.19	2.00	5	0.46	0.16	5	0.11	T
	0.50	7	0.29	1.50	7	0.27	1.56	7	0.09	0.13	7	0.09	C
	0.54	6	0.12	1.02*	6	0.08	1.59*	6	0.22	0.10*	6	0.03	P
	0.88	9	0.17	1.48	9	0.29	1.61	4	0.35	0.75	9	0.48	A
Allatectomized	0.38	5	0.05	1.28	5	0.54	0.99**	5	0.13	0.36	5	0.02	L
	0.05*	5	0.01	0.69**	5	0.21	1.22*	5	0.36	0.06*	5	0.001	T
	0.15	8	0.17	0.62**	8	0.26	1.00	8	0.02	0.03*	8	0.02	C
	0.68	9	0.08	1.37*	9	0.13	1.92**	9	0.20	0.49*	9	0.07	P
Allatectomized	0.90	9	0.19	1.52	9	0.50	1.68	9	0.36	0.69	9	0.12	A
+	0.52	8	0.04	1.78*	8	0.84	1.05	8	0.09	0.47	8	0.09	L
JHa applied	0.15*	9	0.08	1.72**	9	0.32	1.82*	9	0.07	0.12*	9	0.06	T
	0.16	8	0.03	0.99	8	0.27	1.38*	8	0.15	0.15	8	0.05	C

[1] *P*=Protease; *A*=Amylase; *L*=Lipase; *T*=Trypsin; *C*=Chymotrypsin

* = Significant at 5% level; ** = Significant at 1% level

both the cases were found to be significant but the changes appeared more drastic in cauterised and allatectomized individuals where no trace of activity was recorded in fore and hind-gut (table 2). Topical application of juvenoid in allatectomized insects reversed the effects of allatectomy (table 1) and juvenoid applied to the allatectomized plus brain cauterized insects it also produced similar result as obtained in case of allatectomized insects. But the treatment of juvenoid was more effective in case of allatectomized insects than that of the allatectomized plus brain cauterized insects (tables 1 & 2).

Discussion

The results obtained in this study clearly show that there is a qualitative and quantitative fluctuation of digestive enzymes in the gut of *Lohita grandis* during the post-embryonic development which in turn, clearly indicates the changes of food habits or in other words the changes in the major constituents of their food during development. In a previous study Mandal et al. (1981a) suggested that the changes in the quality of food occurred to meet up their physiological needs during the development and probably led to a fluctuation of enzymes level in the gut. This observation corroborates the previous findings of Mandal et al. (1981a) and confirms that like other physiological and behavioural changes the enzymes in the gut also undergo qualitative and quantitative changes during development. (Dahlman 1972, Dadd 1956, Mandal et al. 1981a). The total enzymes activity in the gut reached its peak in adult form, the activity being appreciably high in fifth instar nymph. These two peaks of the enzyme activity clearly signify the stage of maximum

Table 2 *Effect of brain cauterization plus allatectomy and juvenoid treatment on the activity and distribution of five digestive enzymes in the four gut region. Activity expressed as enzyme unit/gut region/min.*

Treatment	Fore-gut (FG)			Mid-gut tissue (MGT)			Mid-gut lumen content (MGC)			Hind-gut (HG)			Enzyme[1]
	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	
	0.74	5	0.05	1.50	5	0.16	2.06	5	0.42	0.50	5	0.02	P
	0.90	8	0.14	1.50	8	0.38	1.75	8	0.09	0.73	8	0.12	A
Control	0.88	5	0.22	2.00	5	0.52	1.83	5	0.33	0.66	5	0.07	L
	0.25	5	0.01	1.75	5	0.19	2.00	5	0.46	0.16	5	0.11	T
	0.50	7	0.29	1.50	7	0.27	1.56	7	0.09	0.13	7	0.09	C
	0.24	4	0.03	0.65**	4	0.02	0.99**	4	0.05	0.02	4	0.01	P
Allatectomized	0.48	4	0.21	1.05*	4	0.23	1.02*	4	0.28	0.45	4	0.12	A
plus	0.23	6	0.10	0.89*	6	0.35	0.49**	6	0.02	0.16	6	0.07	L
Brain-	—	8	—	0.19**	8	0.02	0.32**	8	0.16	—	8	—	T
cauterized	0.02	4	0.02	0.22**	4	0.02	0.51**	4	0.04	—	4	—	C
	0.34*	6	0.07	0.88*	6	0.12	0.99	6	0.02	0.22*	6	0.11	P
Allatectomized	0.48	4	0.28	1.15	4	0.32	0.98	4	0.53	0.45	4	0.12	A
plus	0.27	5	0.02	0.88	5	0.01	0.55*	5	0.03	0.26	5	0.13	L
Brain-	—	7	—	0.22*	7	0.05	0.39	7	0.01	—	7	—	T
cauterized with JHa treated	—	4	—	0.25*	4	0.10	0.51	4	0.12	—	4	—	C

[1] *P*=Protease; *A*=Amylase; *L*=Lipase; *T*=Trypsin; *C*=Chymotrypsin

* = Significant at 5% level; ** = Significant at 1% level

activity of the insect under study (Hori 1972). Rockstein and Kamal (1954) in their comprehensive review work tried to establish the relation between digestive enzyme and the phenomenon of differentiation in insect during development suggested that the physiological modification of insects evolved with changes of morphological adaptations. The qualitative and quantitative fluctuation in the activity of digestive enzymes in the gut fractions as well as in the whole gut during development suggests the probability of differential feeding habit in different stages. With the removal of the corpora allata in adult insects there was a sharp decline in the activity of the digestive enzymes in the gut lumen as well as in the gut tissue which coroborates the findings of Mandal et al. (1981b) in *Schizodactylus monstrosus* and Wigglesworth (1936, 1948) in *Rhodnius prolixus*. The dramatic decline of all enzymes excepting amylase after allatectomy confirms the idea that corpora allata helps in rapid digestion of intestinal content, particularly the protein material (Wigglesworth 1936, 1948). But why the enzyme amylase remains unaffected after allatectomy is not clear. The declining trend of the enzyme activity both in gut lumen and mid-gut tissue (after allatectomy) were much pronounced when allatectomy was coupled with brain-cauterization. It was interesting to note that after removal of both activity of all the enzymes including amylase dropped significantly. Dadd (1961), Thomsen and Moller (1963) and Muraleedharan and Prabhu (1979) reported that after the removal of median neurosecretory cell there was a sharp decline in the activity of enzymes in the gut. But the present study clearly suggests that both corpora allata and neurosecretory cells of brain controls the synthesis and secretion of digestive enzymes, but the secretion of amylase is under the control of neurosecretary cells. This interpretation was further confirmed when juvenoid was topically applied to these operated insects. After the application of juvenoid to the allatectomized insects the enzymes levels in gut increased significantly, the increase being less significant in amylase ($P < 0.05$). But when juvenoid was applied to the allatectomized plus brain-cauterized insects, the enzyme levels also increased, the trend being higher than that of operated insect. But the degree of increase was not so pronounced as in the case of juvenoid applied allatectomized insect. Similar observation was met with in the study of Khan (1964) who suggested that the synthesis and secretion of digestive enzyme is hormonally controlled upon which a secretogogue form of control is imposed. But he failed to specify the hormone responsible for the activity of enzyme. On the basis of present findings it can be concluded that the secretion from corpora allata and brain's neurosecretory cells are necessary for the synthesis and activity of digestive enzymes. But the mechanism by which these two endocrine parts participate in the digestive activity of insects is yet to be determined and elaborate studies are in progress in our laboratory to solve the mystery.

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