STUDIES ON THE DIGESTIVE ENZYMES OF SOME CARNIVOROUS INSECTS

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Hydrogen-ion-concentration in the alimentary canal of *Empusa pauperata*, *Crocothemis servilia servilia* and *Harpactor costalis* ranged between 5.5 to 6.8, 5.5 to 6.5 and 5.8 to 7.5 respectively. In case of first two insects, the contents of the entire alimentary canal and in *Harpactor costalis* Stol, that of mid and hind-gut were weakly acidic, while the secretion of salivary glands and contents of fore-gut in the latter insect were slightly alkaline.

Qualitative analysis of the digestive enzymes showed that carbohydrases were absent in all the parts of the gut but the proteinases (Trypsin and pepsin) were present in fore and mid-gut of all the three test insects. Salivary glands and hind-gut in all cases were devoid of these enzymes.

Quantative estimation of trypsin showed that activity was higher in mid-gut of *Empusa pauperata* and *Crocothemis servilia servilia* than in their fore-gut, whereas, it was reverse in *Harpactor costalis*. The enzyme distribution and activity showed that most of the digestion occurred in fore and mid-gut of these insects.

INTRODUCTION

Studies on the digestive enzymes of insects are of fundamental importance for understanding their food and feeding habits, which can ultimately contribute to successful control of injurious insects. Unfortunately, this aspect has not received due attention and most of the reports are mere speculations based on the type of food taken by the insects. The digestive enzymes of the following entomophagous insects were investigated: *Empusa pauperata* F. (Preying mantis), *Crocothemis servilia servilia* Drury. (Dragon-fly), and *Harpactor costalis* Stol. (Assassin bug).

Hydrogen-ion-concentration (pH) of the alimentary canal provides certain conditions in the medium in which the enzymes act on different food materials. The pH in different gut regions was also studied to find out a correlation between the two.

REVIEW OF LITERATURE

Little work has been done on the digestive enzymes of various insects particularly the entomophagous ones. Bounoure (1919) studied the digestive

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enzymes in Coleoptera and reported that proteases and lipases were predominant in most carnivorous beetles. Edwards (1960) while determining the properties of saliva in assassin bug, *platymeris*, recorded three proteolytic fractions and a phospholipase. In *Corethra*, *Lucilia* and *Glossina*, proteinases, lipases and amylases were recorded from the mid-gut (Gersch, 1952: Hobson, 1931 and Wigglesworth, 1929). The amylase was usually present in the salivary glands of phytophagous and omnivorous insects, whereas only a slight reaction was recorded in the salivary glands of entomophagous insects like *Lucilia* and *Dectlcus* (Hobson, 1931; Schlottke, 1937b). In phytophagous and omnivorous insects most of the proteinases and carbohydrases were active in the fore and mid-gut usually; being perhaps the primary seat of digestion in these insects. In entomophagous insects this type of activity appears to be prevalent only in the mid-gut.

The Hydrogen-ion-concentration in the alimentary canal of various insects was studied by different workers. As far as the entomophagous insects are concerned, Ballentine (1940), Shinoda (1930) and Swingle (1931) reported that pH in the alimentary canal of Anisopterous nymphs, *Anax*, *Libellula luctuosa*, assassin bug and *Vespula* ranged from 5.0 to 8.0, 6.8 to 7.2, 5.4 to 6.3, 5.5 to 7.3 and 5.3 to 6.1, respectively.

MATERIALS AND METHODS

Adult insects were collected from the field according to the requirements every time for all the experiments. The experimental insects were starved for 24 hours and were then fed on distilled water for an hour to clear their gut contents.

Determination of hydrogen-ion-concentration in different gut regions

Ten individuals of each species were dissected, their alimentary canals removed and divided into three portions: fore-gut, mid-gut and hind-gut. Salivary glands were also removed. The homogenates of salivary glands and portions of guts were prepared by macerating in separate watch glasses. The pH was determined by applying Universal Indicator Paper strips to the homogenates and comparing the colour of the moistened papers with B.D.H. Standard Chart.

Preparation of enzyme extract

The alimentary canal and salivary glands of ten individuals of each species were dissected in Bodenstein's Insect Ringer solution. Each alimentary canal was divided into three portions and macerated for 5 minutes after adding 1 ml. distilled water in each case. These tissue homogenates were centrifuged for

10 minutes, after making the volume up to 5 ml. with distilled water, in separate tubes. The supernatant was used for enzyme determination.

Qualitative determination of some important digestive enzymes

Enzyme extract (0.5 ml.) along with substrate (0.5 ml.) and buffer solution (0.25 ml.) was incubated at 34°C. for 4 hours. The proteinases and carbohydrases were detected by subjecting the incubated mixture to Nelson's technique (Nelson, 1944) and chromatographic technique (Smith, 1960).

Quantitative estimation of trypsin

One ml. of enzyme extract was added to an equal volume of each of the substrate and buffer solution and then incubated for two hours at 34°C. After incubation, 8 ml. of 5 per cent trichloroacetic acid was added and precipitated protein was separated by filtration. To a 5 ml. aliquot of filtrate was added an equal volume of 0.5 N sodium hydroxide and light transmission and optical density were recorded at mll 450 using Unicam spectrophotometer. The velocity constant of enzyme activity was calculated as expressed by Charney and Tomarelli (1948) in the following equation:

$$k = \frac{1}{t} 2.3 \log \frac{C_1}{C_2}, \text{ where}$$

t = time in minutes,
C_1 = Initial reading, and
C_2 = Final protein concentration.

Optical density values were substituted for 'C' values. The initial concentration (C_1) was determined by adding 5 ml. of 0.5 N sodium hydroxide to 5 ml. of substrate (diluted twenty-times with distilled water) and 1 ml. each of enzyme extract and buffer solution and then reading the colour of the final solution. The C_2 value was determined by subtracting the optical density of the trichloroacetic acid filtrate from C_1 value. The optical density in case of initial protein concentration was multiplied by 20 to get the correct C_1 value because it was already diluted 20 times. The calculated K value was multiplied by 10^5 to get whole numbers for ease of calculations. These values were analysed statistically.

RESULTS AND DISCUSSION

Hydrogen-ion-concentration (pH) in different portions of the alimentary canal was recorded and the results are given in Table 1. The pH in the alimentary canal of *Empusa pauperata* F., *Crocothemis servilia servilia* Drury and *Harpactor costalis* Stol ranged from 5.5 to 6.8, 5.5 to 6.5 and 5.8 to 7.5

respectively. These observations correspond to those of Swingle (1931) on a dragon-fly, *Libellula luctuosa* and a species of an Assassin bug in which the pH range of the alimentary canal was 5.4 to 6.3 and 5.5 to 7.3 respectively. But these differ from the observations of Ballentine (1940) and Shinoda (1930) who reported a pH range of 5.0 to 9.0 and 6.8 to 7.2 in case of Anisopterous nymph and *Anax*. However, the trend was that the pH decreased in the mid-gut and again there was an increase in the hind-gut and in general the contents of the alimentary canal were weakly acidic.

TABLE 1.—*pH range in different gut regions.*

Name of insect	Part of alimentary canal	pH range	Average
Empusa pauperata	Salivary glands	6.5—6.7	6.57
	Fore-gut	6.5—6.8	6.63
	Mid-gut	5.5—5.6	5.57
	Hind-gut	6.0—6.5	6.30
Crocothemis servilia servilia	Salivary glands	5.5—5.8	5.63
	Fore-gut	5.6—5.8	5.67
	Mid-gut	5.5—5.6	5.57
	Hind-gut	5.8—6.5	6.10
Harpactor costalis	Salivary glands	7.2—7.4	7.33
	Fore-gut	7.3—7.5	7.40
	Mid-gut	5.8—6.0	5.90
	Hind-gut	6.0—6.4	6.23

Studies on enzymes (Table 2) showed that carbohydrases (Amylase, invertase and maltase) were absent in all the three insects under experiment, while proteinases (trypsin and pepsin) were present in the fore-gut and mid-gut. This indicates that these insects are able to hydrolyse only the protein constituents of their diet or they live on proteinous food only. These results are in line with the observations of Gersch (1952), Hobson (1931) and Schlottke (1937 a, b) who recorded a general absence of carbohydrases in the alimentary canal and presence of only the proteinases in the mid-gut of *Corethra* larva, *Lucilia* larva, carabid beetles and predatory *Decticus*.

On the other hand, the occurrence of trypsin and pepsin in the fore-gut and mid-gut of the test insects also corresponds with the findings of phytophagous and omnivorous insects, where these enzymes are usually met with in the same region in combination with carbohydrases, as for example, *Cecva*

TABLE 2.—*Occurrence of enzymes in different gut regions.*

Name of insect	Enzymes	Salivary glands	Fore-gut	Mid-gut	Hind-gut
Empusa pauperata	*Proteinases*				
	Trypsin	—	+	+	—
	Pepsin	—	+	+	—
	Carbohydrases				
	Amylase	—	—	—	—
	Invertase	—	—	—	—
	Maltase	—	—	—	—
Crocothemis servilia Servilia	*Proteinases*				
	Trypsin	—	+	+	—
	Pepsin	—	+	+	—
	Carbohydrases				
	Amylase	—	—	—	—
	Invertase	—	—	—	—
	Maltase	—	—	—	—
Harpactor costalis	*Proteinases*				
	Trypsin	—	+	+	—
	Pepsin	—	+	+	—
	Carbohydrases				
	Amylase	—	—	—	—
	Invertase	—	—	—	—
	Maltase	—	—	—	—

+ Shows the presence of enzymes.
— Shows the absence of enzymes.

capsid, *Dysdercus koenigii*, *Locusta migratoria*, *Ostrinia nubilalis*, *Blattella germanica*, *Periplaneta americana*, and *Acrotelsa collaris* (Goodchild, 1952; Saxena, 1958; Khan, 1963; Bottger, 1940; Wigglesworth, 1927-1928; Swingle, 1925; and Modder, 1964). However, the presence of a weak amylase in the salivary glands of *Lucilia* larva (Hobson, 1931) and predatory *Dectlcus* (Schlottke, 1937b) cannot be refuted because it is just possible that the insects under investigation may have an amylase or any other carbohydrases too weak to be detected. But the absence of enzymes from the contents of hind-gut is well established. Schlottke (1937a) has very clearly indicated that the activity of enzymes diminishes towards the end of the intestine, being practically absent from the hind-gut.

The activity of trypsin was recorded in the test insects and the results are represented in Fig. 1. The activity of trypsin was significantly higher in the mid-gut of *Crocothemis servilia servilia* Drury and *Empusa pauperata* F. than in their fore-gut. This observation corresponds to the findings of Schlottke (1937a)

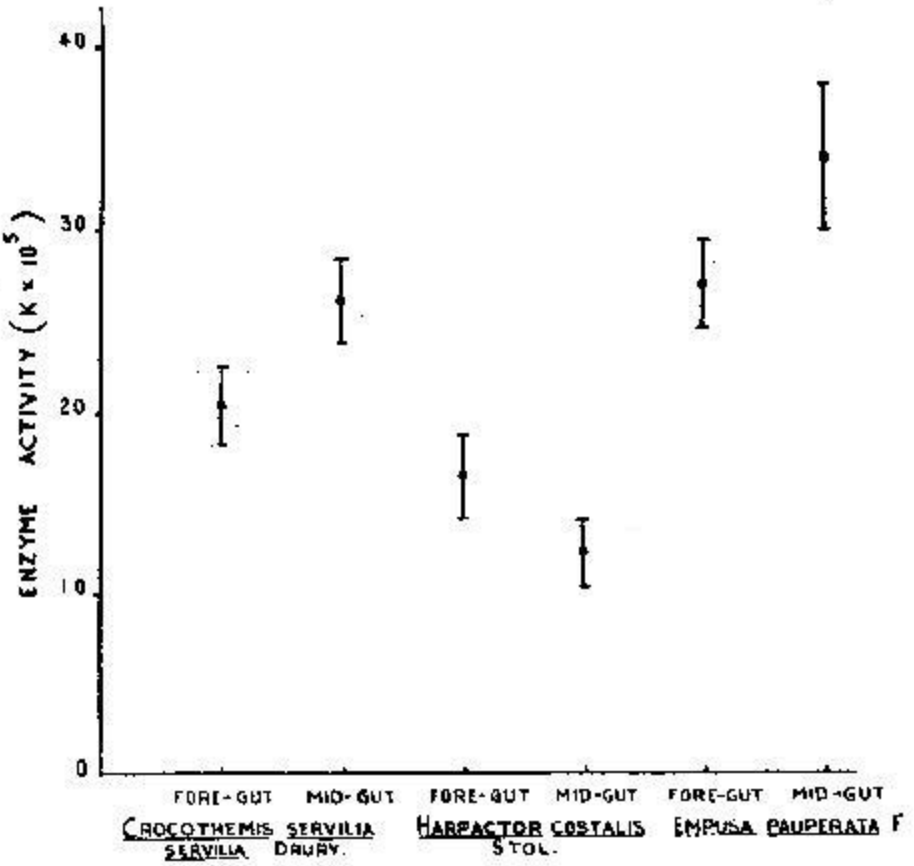

Fig. 1 Activity of trypsin in test insectes.

with regard to carabid beetles where the enzymes were found to increase in quantity in the mid-gut. Wigglesworth (1929) noticed a marked contrast in enzymatic activity of *Calliphora* and *Glossina* as in the latter protease was highly active in the mid-gut and carbohydrases were absent excepting a very feeble amylase activity in the same region, whereas, in the former amylase, invertase and maltase were highly active in the mid-gut with only a light activity of protease. This difference is obviously due to the difference in the nature of food of the two insects; *Calliphora* feeding largely on sweet substances and *Glossina* feeding exclusively on blood. This can, however, be

explained by quoting the example of *Chrysops* which feeds on both blood and nectar and occupies an intermediate position. In this case an active protease and invertase and a weak amylase were present in the mid-gut (Wigglesworth, 1931).

A higher activity of trypsin in the fore-gut of *Harpactor costalis* in the present investigations is interesting and no speculations can be made in this case due to lack of published work in this direction. However, a comparative study of predators of different groups as well as of different species of the same genus may prove useful.

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