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Studies on the Physiology of Digestion in the last Instar Larva of the Rice Moth (Corcyra cephalonica Stainton)

II. Digestive enzymes and effect of starvation on their secretion

(*Lepidoptera*:*Galleriidae*)

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Introduction

SRIVASTAVA (1960) studied the hydrogen-ion concentration of the gut of the larva of *Corcyra cephalonica* STAINTON. The present paper deals with the various digestive enzymes secreted by the alimentary canal of the larva and also the effect of starvation on the secretion of these enzymes.

Material and methods

Larvae were reared in the laboratory as already described (SRIVASTAVA, 1960). Fully developed larvae of the last instar were taken out from the culture for each set of experiment. They were equally divided in two sets. Estimations were made immediately from one set of the larvae, while the larvae of the other set were starved individually for a period of five days in small petri dishes, and their excreta cleaned four times daily in order to avoid its ingestion. No larva died because of this short starvation during the course of experiment.

Homogenate was prepared in ice-cold double distilled water, as glycerol had inhibitory effect on some of these enzymes (SRIVASTAVA, 1959). Ten larvae were taken at a time for the preparation of the homogenate, they were dissected in ice-cold distilled water. Alimentary canal after being cleaned with the adhering tissues was divided into

[1]) Part of the thesis approved for the degree of Doctor of Philosophy of the Lucknow University in 1958.

DOI: 10.21248/contrib.entomol.11.1-2.11-15

five pieces, viz., fore-gut, mid-gut, ileum and colon, rectum and Malpighian tubules. The contents and the tissues of these pieces were separately stored in watch glasses containig a little distilled water and a drop of toluene, till all the ten larvae had been dissected. These watch glasses were maintained at sufficiently low temperature by keeping them in a glass trough filled with freezing mixture. Tissues were separately homogenized in an electrically operated „Pyrex tissue grinder", while the contents were simply teased or beaten so as to get a homogenous suspension. The volume of the homogenate in each case was made to 10 ml. in a volumetric flask. Homogenate thus prepared was stored in a refrigerator for 1½ hours, centrifuged and supernatant used for enzyme assays. Enzymes in the homogenate to be used in control tubes were denatured by immersing them in boiling water for 15 minutes.

Methods given in Cole's (1942) and Hawk, Oser & Summerson's (1947) books were employed for the detection of various digestive enzymes except the proteases, for which the technique was slightly modified to suit the present investigations. Coagulated egg albumin filled in glass capillary tubes was given as substrate. Small pieces of these capillary tubes were suspended into the reaction mixture, and the presence of enzyme was indicated, when the albumin was digested only in the experimental tube leaving behind the empty capillary tube. Incubation was carried out at 37° C. For amylase, invertase, lactase and maltase reaction mixtures were incubated for one hour, for lipase 12 to 24 hours, for trypsin and pepsin 24 to 48 hours and for cellulase 2 to 7 days. Optimum pH as determined by Srivastava (1960) for amylase, invertase, lipase and trypsin was maintained during the reaction of these enzymes, while pH in the case of lactase and maltase was kept at 6.5, for pepsin at 3.0 and for cellulase 7.5.

Discussion

Secretion of enzyme amylase, invertase, maltase, lipase and protease (trypsin) from the mid-gut epithelium indicate that the larvae of *C. cephalonica* like other lepidopterous larvae studied by Stober (1927), Shinoda (1930), Schlottke (1944) and Srivastava (1955) are able to utilize all the essential constituents of the food, viz., carbohydrates, proteins and fats.

The presence of certain enzymes in the contents of the fore-gut of *C. cephalonica* larvae and their complete absence in its wall clearly indicate that these enzymes are not secreted in this region of the alimentary canal, but are positively regurgitated from the mid-gut. This is in agreement with the findings of Abbott (1926) and Day & Powning (1949).

Persistant presence of only amylase and invertase in the larvae of *C. cephalonica* starved for a period of 5 days leads author to believe that these two enzymes do not require the stimulus of feeding for their secretion, while others, viz., maltase, lipase and trypsin do require the the stimulus of feeding for their secretion, as these are ceased to be secreted during starvation. The present observations regarding the stimulus of secretion is in conformity with those of Fisk & Shambaugh (1952) on *Aedes aegypti* L. and Saxena (1955) on *Dysdercus koenigii* Fabr. It has been reported by Fisk & Shambaugh (1952) that the residual protease activity becomes very low in blood starved *A. aegypti*, and that the stimulus of feeding of blood is required for its secretion. They found that contrary to the behaviour of protease there is a significant activity of invertase in the tissues of the mid-gut and diverticulae of the starved mosquitoes. Fisk & Sham-

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Observations

Table 1. Table showing the distribution of various digestive enzymes in the different regions of the alimentary canal of *Corcyra cephalonica* larva

Enzymes	Regions of the gut								
	Fore-gut		Mid-gut		Ileum and colon		Rectum		Mal-pighian tubules
	Wall	Contents	Wall	Contents	Wall	Contents	Wall	Contents	
Amylase	—	+	++	+++	—	—	—	—	—
Invertase	—	+	++	+++	—	—	—	—	—
Lactase	—	—	—	—	—	—	—	—	—
Maltase	—	—	+	++	—	—	—	—	—
Lipase	—	—	+	++	—	—	—	—	—
Trypsin	—	—	++	+++	—	—	—	—	—
Pepsin	—	—	—	—	—	—	—	—	—
Cellulase	—	—	—	—	—	—	—	—	—

Table 2. Table showing the distribution of various digestive enzymes in the different regions of the alimentary canal of 5 days starved *Corcyra cephalonica* larva.

Enzymes	Regions of the gut								
	Fore-gut		Mid-gut		Ileum and colon		Rectum		Mal-pighian tubules
	Wall	Contents	Wall	Contents	Wall	Contents	Wall	Contents	
Amylase	—	+	+	+	—	*	—	—	—
Invertase	—	+	+	+	—	—	—	—	—
Lactase	—	—	—	—	—	—	—	—	—
Maltase	—	—	—	—	—	—	—	—	—
Lipase	—	—	—	—	—	—	—	—	—
Trypsin	—	—	—	—	—	—	—	—	—
Pepsin	—	—	—	—	—	—	—	—	—
Cellulase	—	—	—	—	—	—	—	—	—

\+ indicates positive but weak enzymatic activity,
++ indicate strong enzymatic activity,
+++ indicate very strong enzymatic activity,
* indicates occasional enzymatic activity,
— indicates no enzymatic activity

BAUGH (1952) are in the favour of "secretogouge" theory, while DAY & POWNING (1949) maintain that hormones provide stimulus of secretion, they have also mentioned that protease (trypsin) is present in the gut of three days starved cockroach.

Occasional presence of the enzyme amylase in the contents of the ileum and colon of the starved larvae is interesting and calls for comments. The absence of the enzyme amylase in the contents of the rectum clearly indicates its disposal in the region of ileum and colon. It is quite possible

DOI: 10.21248/contrib.entomol.11.1-2.11-15

that finding no substrate to react in the region of its secretion (i. e., mid-gut) it makes its way into the ileum and colon, from here it is probably absorbed into the haemolymph; as it does not descend further to be thrown out with the excreta.

Acknowledgments

The author is thankful to Dr. P. D. Gupta for guidance, to Professor M. B. Lal for the provision of the laboratory facilities and to the Government of India for the awrad of a Research Training Scholarship.

Summary

Out of the entire alimentary canal the mid-gut epithelium is the only part which secretes digestive enzymes amylase, invertase, maltase, lipase and trypsin, in the larva of *Corcyra cephalonica* Stainton. Enzymes are regurgitated even during starvation, though the secretion of some of these enzymes is stopped in the larvae starved for 5 days. Stimulus of feeding is required for the secretion of maltase, lipase and trypsin, where as amylase and invertase do not need the stimulus of feeding for their secretion.

Zusammenfassung

Bei der Larve von *Corcyra cephalonica* Stainton ist im gesamten Verdauungskanal das Mitteldarmepithel der einzige Abschnitt, der Verdauungsfermente sezerniert (Amylase, Invertase, Maltase, Lipase und Trypsin). Enzyme werden sogar während des Hungerns abgegeben, obgleich die Sekretion einiger dieser Enzyme bei Larven nach 5tägigem Hungern eingestellt wird. Eine stimulierende Wirkung der Nahrung ist erforderlich für die Sekretion von Maltase, Lipase und Trypsin, während Amylase und Invertase zu ihrer Sekretion einer Stimulation durch die Nahrung nicht bedürfen.

Резюме

У личинки *Corcyra cephalonica* Stainton эпителий средней кишки во всем прищеварительном тракте является единственным участком, выделяющим ферменты пищеварения (амилазу, инвертазу, малтазу, липазу и трипсин). Энзимы выделяются даже во время голодования, хотя выделение некоторых из этих энзимов личинок прекращается после пятидневного срока голодания. Стимулирующее действие пищи необходимо для выделения малтазы, липазы и трипсина, между тем, как амилаза и инвертаза не нуждаются в пище для их выделения.

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On the Hydrogen-Ion Concentration in the Alimentary Canal of the Coleoptera

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Introduction

In an earlier paper on the hydrogen-ion concentration of certain Orthopteroid insects, the authors (Srivastava & Srivastava, 1956) had concluded that within a particular group of insects, the hydrogen-ion concentration of the different parts of the alimentary canal shows a limited range within which the variation observed from insect to insect may be related to the feeding habit. It was also shown that while the difference in the hydrogen-ion concentration of the various kinds of food may affect the hydrogen-ion concentration of the fore-gut and the hind-gut of an insect, it does not influence the hydrogen-ion concentration of the mid-gut which shows a great deal of constancy. These conclusions were based on a study of the hydrogen-ion concentration of 15 species of relatively primitive groups, and it appears necessary to check their validity by observations in some other order, preferably a highly evolved one. In the present paper, therefore, the authors have given the hydrogen-ion concentration of the different parts of the alimentary canal of 16 species of *Coleoptera* belonging to 12 families and with diverse food habits. In some cases, both larvae and adults of the same species were examined.

Material and technique

The names of the insects examined, along with their families and food habits, have been given in Table 1. The method of determining the pH was broadly the same as described before (Srivastava & Srivastava, 1956). But in certain cases, efforts to feed the insects on bits of pH paper have succeeded. In these cases, the alimentary

DOI: 10.21248/contrib.entomol.11.1-2.11-15