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The Digestive Enzymes in the Larvae of *Chaoborus obscuripes* VAN DER WULP, 1867

With 1 text figure

There are numerous works on the digestive enzymes and the physiology of digestion in insects. WIGGLESWORTH (1927) and SCHLOTTKE (1937) carried out detailed surveys regarding the process of digestion and the enzyme complements in cockroach. The pertinent literature on the physiology of digestion in insects was reviewed by UVAROV (1928) and by WATERHOUSE (1957), while TATCHELL (1958) reviewed the literature pertaining to Diptera.

In view of the great variety of food and feeding habits encountered among the insects more extensive work is necessary to give us a complete picture of the process of digestion in this group as a whole. The present work has been carried out as part of a more comprehensive study of the role of the mid-gut in digestion in the larvae of *Chaoborus obscuripes* and is probably the first of its nature. Earlier observations by MONCHADSKY (1945) confirmed by histological and in vivo studies by SCHÖNFELD (1958) and CHOWDHURY (in press) showed that in these fresh-water predacious larvae the digestive enzymes are produced in the mid-gut and propelled, at least partially, to the crop by means of antiperistalsis. The prey, comprising of *Daphnia*, *Cyclops* and other smaller aquatic arthropods, are swallowed whole and reach the crop. Here a partial digestion takes place and liquified nutrient material only passes on to the mid-gut, where the process of digestion is completed. On the basis of these results of the study of the digestive enzymes in *Chaoborus obscuripes* larvae the crop and the mid-gut only have been considered.

Material

Full grown larvae of *Chaoborus obscuripes* were collected and cultured in the laboratory on a diet of *Cyclops* and *Aëdes* larvae. These larvae failed to pupate when kept at a temperature of about 5 °C. However, to ensure normal physiological conditions, all larvae used in experimental work were kept at room temperature (20 °C ± 1 °C) for not less than four days before being used.

The "starved" larvae mentioned throughout this paper are those that were kept without food for four days before the experiment, and the "fed" larvae are those that were fed 1 to $1^1/_2$ hours before the experiment following a four-day starvation.

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Preparation of Enzyme Material

For enzyme preparations larvae were dissected in cold distilled water, and the crop and mid-gut were separated. While the crops were immediately collected in 2 or 3 drops of the required buffer solution the mid-guts were cut into 3 or 4 pieces, the contents gently washed out in normal saline and the tissues then removed to 2 or 3 drops of the buffer solution. The crops and the mid-guts were homogenised separately in a 1 ml glass homogeniser and the homogenates washed into separate test tubes with the required quantity of the buffer. The contents were simply washed into another test tube with the buffer solution. A few drops of toluol were added in every case to stop bacterial growth. Use of glycerol extracts was avoided since SRIVASTAVA (1959) had shown that 50 per cent glycerol had a definite inhibitory effect on amylase, invertase and lipase. The number of mid-guts and/or crops, the quantity of buffer solution and the period of incubation in each case had been selected after preliminary studies. Incubation was carried out at a temperature of 38 °C ± 0.5 °C (38 °C and 40 °C for lipase and 40 °C for dipeptidase). Controls were carried out in each case using plain buffer instead of the homogenate.

Phosphate buffer made up of 0.15 M Na_2HPO_4 and 0.15 M KH_2PO_4 was used in the analysis of all enzymes except acid and alkaline phosphatase. For these HCl-Na-Acetate and Borate-HCl buffers respectively were used. Methods given by COLE (1955) were used for various qualitative analyses.

Results

Proteinase: Preliminary experiments on proteinase activity were carried out using photo-plates which had been already exposed, developed and fixed. These were washed and stored dry. Drops of homogenate in buffers of different pH ranging from 2.0 to 8.5 were placed alongside with plain buffer drops. The plates were then incubated at 38 °C in a moist chamber for different lengths of time (15 minutes to $3^1/_2$ hours). After incubation the plates were allowed to cool down to room temperature and then gently washed in a pan of cold water. It was assumed that enzyme activity had taken place when gelatin from the areas where drops of homogenate were placed washed out leaving gelatin-free glass behind, because this showed that gelatin had been dissolved by the action of proteinase. These preliminary experiments clearly indicated the presence of proteinase in mid-gut tissue, mid-gut contents and crop of both starved and fed larvae.

Quantitative analysis of proteinase activity to determine the optimal pH for proteinase was carried out by incubating the homogenate prepared in buffer solutions with pH ranging from 2.5 to 8.5 and 5 per cent gelatin as substrate. In each case 20 crops and mid-guts from starved larvae in 1.5 ml of the buffer solution and 2 ml of the substrate were used and incubation was carried out for 44 hours. After incubation formalin titration to pH 9.2 was carried out. A LPH-01 type pH-meter was used for titration to ensure accurate values. The results are presented graphically (Fig.). The graph plotted shows a single peak at pH 7.5. No proteinase activity was detectable at pH 2.5 and very low activity at pH 3.0 and 4.0. A comparison of fed and starved larvae showed higher proteinase activity in the former.

Dipeptidase: 2 ml of 2 per cent glycil-glycine was used as a substrate. 25 larvae and 1 ml of the buffer at pH 7.5 were used in this case. Incubation period was 3 hours, the temperature being 40 °C. Formalin titration to pH 9.2 using a pH-meter was carried out at the end of incubation. Dipeptidase was detected in the crop, mid-gut contents and mid-gut tissue of fed larvae only, being totally absent in the starved ones.

Carbohydrases: Qualitative tests for detection of amylase, invertase, maltase and lactase were carried out. Homogenates of crop, mid-gut tissue and mid-gut contents from 20 larvae in 1 ml of buffer solution (pH — 6.5) were used in each case.

Amylase was detected in crop, mid-gut tissue and contents of both fed and starved larvae. 2 drops of 0. 5 per cent starch solution were used as substrate. Presence of amylase was concluded when Lugol's iodine test gave negative results. Incubation for one hour was found sufficient for the complete breakdown of starch.

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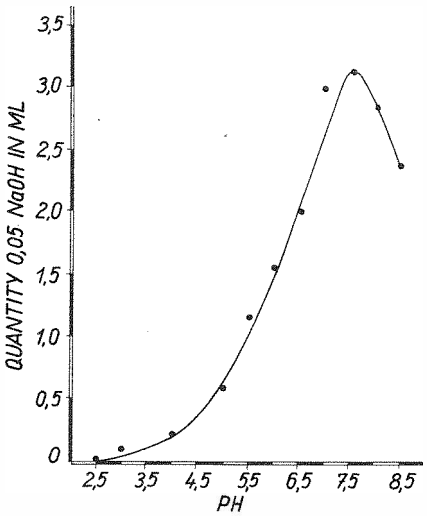

Effect of pH on Proteinase Activity

For invertase 0.1 ml of 3 per cent sucrose solution and for maltase 0.2 ml of 3 per cent maltose solution were used as substrates. Incubation was carried out for two hours in each case. Trommer's test and osazone test were carried out for detecting invertase and maltase. respectively. These two enzymes were detected in the mid-gut tissue of fed as well as starved larvae while the crop and the mid-gut contents were found to contain no invertase or maltase.

In case of lactase 0.2 ml of 3 per cent lactose served as the substrate and the incubation period was two hours. Lactase activity was not detected either in the mid-gut or in the crop. Osazone test was employed in this case also.

Since these experiments showed that maltase was present in the mid-gut tissue only whereas amylase was present in the mid-gut tissue, mid-gut contents as well as in the crop, it was decided to determine the final breakdown products of starch under the action of amylase alone. For this, homogenates of crops, mid-gut tissue and mid-gut contents from 40 larvae, one and a half hour after feeding, were prepared in 2 ml of the buffer at pH 6.5. These were separately incubated with 0.4 ml of 3 per cent starch solution each for four hours. Lugol's iodine test and osazone test were carried out on equal portions of the incubate. Iodine tests were negative in every case, showing that starch had been broken down. But while osazone test with the incubates containing mid-gut tissue homogenate showed presence of glucose, the homogenates of crop and the mid-gut contents showed the presence of maltose only.

Phosphatases: Homogenates of crops, mid-gut tissue and mid-gut contents from 40 larvae in 1 ml of the required buffer at pH 8.6 for alkaline phosphatase and at pH 5.0 for acid phosphatase were used in each case. 1 ml of 1 per cent Na-glycerophosphate served as the substrate. Incubation was carried out for 24 hours. Tests for the presence of phosphate using amonium molybdate were conducted at the end of incubation. Both acid and alkaline phosphatases were detected in the crop, mid-gut tissue and mid-gut contents of fed as well as starved larvae.

Lipase: Olive oil and sunflower seed oil as substrates and buffer solutions at pH 7.5 and 7.8 were tried for lipase. Emulsions were prepared by shaking 2 ml of pepsin with 1 ml of the substrate or by the method of WILLSTATTER, WALDSCHMITT-LEITZ and MEMMEN. Incubations at 38 °C for 48 hours were carried out. No lipase activity could be detected.

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Discussion

Of the nine different enzymes examined, all except lactase and lipase were found to be produced by the mid-gut epithelium. The results of the enzyme analyses are presented in table. Similar observations were reported, among others, by ABBOTT (1926), DAY & POWNING (1949), SRIVASTAVA (1961) and KHATOON (1967). In this respect no functional division could be made between the different sections of the mid-gut, because the entire mid-gut is morphologically as well as histologically uniform, SCHÖNFELD (1958), CHOWDHURY (in press), and an attempt at subdividing it would be a matter of random choice only.

Table
Results of enzyme analyses

Enzymes	Crop		Mid-gut			
			Contents		Tissue	
	Starved	Fed	Starved	Fed	Starved	Fed
Proteinase	+	++	+	++	++	+++
Dipeptidase	–	+	–	++	–	++
Amylase	+	+	++	+++	++	+++
Invertase	–	–	–	–	+	++
Maltase	–	–	–	–	+	++
Lactase	–	–	–	–	–	–
Acid Phosphatase	+	++	+	++	++	+++
Alkaline Phosphatase	+	++	+	++	++	+++
Lipase	–	–	–	–	–	–

All the enzymes except dipeptidase were present either in the mid-gut tissue and/or mid-gut cavity of the starved as well as the fed larvae. This clearly indicates that except for dipeptidase a feeding stimulus is not essential for the production and secretion of the enzymes. However, it was noted that there was a definite increase in the quantity of enzyme after feeding. This had been proved by quantitative analysis in case of proteinase. This lends support to the histological findings of the present author (in press) to the effect that secretion droplets are formed in the starved larvae as well. DAY & POWNING (1949) also noted the presence of a trypsin-like proteinase in the gut of cockroaches starved for three days. But SAXENA (1955) in *Dysdercus koenigi* and SRIVASTAVA (1961) in *Corcyra cephalonica* reported a feeding stimulus to be essential for the secretion of maltase, lipase and trypsin whereas invertase and amylase were detected by these authors in the starved ones as well.

The proteinase found in *Chaoborus obscuripes* larvae showed a single sharp peak at pH 7.5 and a very wide pH range in which it was active. It was interesting to note that hardly any proteinase activity was detected at pH 3.0 which means that pepsin-like proteinase was absent. HUNT & MOORE (1958) had shown that there is a complex protease system in bacteria. They obtained a single well-defined peak in a plot of hydrolysis versus pH, but found three separate peaks from electrophoretic separation of the same enzyme complex.

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Three electrophoretically separate trypsin-like substances had also been reported by PATTERSON & FISK (1958) in *Stomoxys calcitrans* and by PATEL & RICHARDS (1960) in *Musca domestica*. It is quite probable that in *Chaoborus obscuripes* larvae also a number of proteases rather than a single one is involved in the breakdown of gelatin.

Dipeptidase activity at pH 7.5 had been detected in fed larvae only. This seems interesting in view of the presence of a ferment-chain for the digestion of proteins as suggested by SCHLOTTKE (1937). It is possible that the animal protein on which these larvae feed is first broken down to di-and tripeptide stage by the trypsin-like enzyme system and then these, in their turn, act as substrates for dipeptidase action ultimately releasing the essential amino-acids.

Among the carbohydrases examined amylase, invertase and maltase had been detected. All these had been found in fed and starved larvae. The absence of lactase in these larvae agrees with the observations of HOBSON (1931), EVANS & MARSDEN (1956), TATCHELL (1958), SRIVASTAVA (1961) and others.

While amylase was found in the crop, mid-gut contents and mid-gut tissue, invertase and maltase were found in the mid-gut tissue only. This should mean that the last two are never poured into the trophic cavity. They are localised either inside or on the gut-surface of the epithelial cells. Experiments further showed that the amylase was not capable of breaking down polysaccharides completely, i.e. to monosaccharides. The amylase breaks down starch to disaccharides only and the latter under the influence of maltase break down to glucose. But maltase was never found in the gut cavity or crop where amylase was active. This is another example of successive action by a group of enzymes. The presence of all these carbohydrate splitting enzymes in these predacious larvae does not support the suggestion that insects feeding on a protein-rich diet have consequently lost the carbohydrate digesting enzymes or are at least poor in them (DAY & WATERHOUSE 1953). EVANS (1956, 1958) also showed that the larvae of *Calliphora erythrocephala* and *Lucilia sericata* possess a very wide range of carbohydrases in spite of the fact that both these larvae are carnivorous. Quite understandably, the presence of these various carbohydrases enables these larvae to utilize the animal food more completely, not depending on the protein part of their food alone for their energy requirements.

Acid phosphatase active at pH 5.0 and alkaline phosphatase active at pH 8.6 had been detected. In earlier literature records of an acid phosphatase in insects are scanty. Among more recent works KHATOON (1967) reported the absence of acid phosphatase in *Utetheisa pulchella* larvae. The finding of definite acid phosphatase activity in *Chaoborus obscuripes* larvae is further interesting in view of the fact that GERSCH (1952) reported that in *Chaoborus* larvae the gut pH varies from 6.2 to 8.8. This anomaly may be explained by considering the variation in acidity conditions as a direct result of feeding, particularly when, as in the case of these larvae, the food consists of whole animals. Presence of enzymes in the prey may increase the acidity. The presence of protease active

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in such highly acid conditions as pH 3.0 and 4.0 further confirms this observation.

The absence of a lipase in these predacious larvae at first seems to be striking. However, absence of a lipase capable of splitting olive oil and sunflower seed oil should not be taken as a proof of the total lack of fat digesting enzymes. TATCHELL (1958) found that the lipase in the parasitic larvae of *Gasterophilous intestinalis* was incapable of splitting olive oil and ethylbutyrate while definite lipase action was detected when tributyrin was used as a substrate. FODOR (1948) showed that the cleavage of lower and higher glycerides is not performed by the same enzymatic component. He reported a complex lipase system consisting of three components, each of which had its own optimal pH and was capable of splitting either olive oil or methyl butyrate or monobutyrine.

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Summary

The present paper reports the findings of a study of nine different enzymes produced by the mid-gut of *Chaoborus obscuripes* larvae in relation to feeding. Of the enzymes studied proteinase, amylase and acid and alkaline phosphatases were found in the mid-gut cavity and mid-gut tissue of fed as well as starved larvae. Invertase and maltase were detected in the mid-gut tissue of both fed and starved larvae, but never in the trophic cavity. Dipeptidase was detected in the mid-gut cavity and mid-gut tissue of fed larvae only. Lactase and lipase (capable of breaking down olive oil and sunflower seed oil) were found to be absent. — The author concludes that the predacious habit has not caused a reduction of the carbohydrate digesting enzymes and that a ferment-chain system is operating in these larvae whereby a series of enzymes, in succession, break down the complex food substances step by step.

Zusammenfassung

Dieser Artikel enthält die Ergebnisse einer Untersuchung von neun verschiedenen Enzymen, die vom Mitteldarm von *Chaoborus obscuripes*-Larven bei der Nahrungsaufnahme erzeugt werden. Von diesen Enzymen wurden Proteinase, Amylase sowie saure und alkalische Phosphatasen in der Mitteldarmhöhle und im Mitteldarmgewebe gefütterter wie ungefütterter Larven festgestellt. Invertase und Maltase fanden sich im Mitteldarmgewebe gefütterter wie ungefütterter Larven, aber niemals im Nahrungsraum. Dipeptidase wurde nur bei gefütterten Larven in der Mitteldarmhöhle und im Mitteldarmgewebe gefunden. Laktase und Lipase (die Olivenöl und Sonnenblumenkernöl abbauen können) wurden nicht festgestellt. — Der Autor folgert, daß die räuberische Lebensweise nicht zu einer Verringerung der Kohlehydrate abbauenden Enzyme geführt hat und daß bei diesen Larven ein Fermentkettensystem wirksam ist, in dem eine Reihe von Enzymen nacheinander komplexe Nahrungsmittel schrittweise abbauen.

Резюме

Изучались девять разных ферментов, вырабатывающихся в средней кишке личинок комара *Chaoborus obscuripes* в связи с питанием. Из изученных ферментов протеаза, амилаза, а также кислая и щелочная фосфатазы были найдены в полости и ткани средней кишки как у сытых так и у голодных личинок. Инвертаза и мальтаза были найдены только в ткани средней кишки

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голодных и сытых личинок, но не обнаруживались в её полости. Дипептидаза найдена в полости и в ткани средней кишки только у сытых личинок. Лактаза и липаза (расщепляющая оливковое и кукурузное масла) вообще не обнаружены. — В заключение автор сделает следующие выводы: Хищный образ жизни этих личинок не уменшало количество и набор карбогидразорасщепляющих ферментов. У данных личинок действует система ферментативной цепи. Каждый фермент этой цепи действуя в определенном порядке постепенно расщепляет сложные пищевые вещества.

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