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The digestive enzymes of the larva of *Utetheisa pulchella* LINNAEUS

(Lepidoptera: Arctidae)

With 2 textfigures

Introduction

Although there is a mass of information on the occurrence of the digestive enzymes in a variety of insects which have been reviewed by UVAROV (1928), WIGGLESWORTH (1953), DAY & WATERHOUSE (1953) and WATERHOUSE (1957). But, however, in insects with regard to the diversity in the mechanism of feeding, food selection and feeding behavior etc. the data is still fragmentary as compared to those on vertebrates, to formulate well defined theories on the physiology of digestion. Therefore, further detailed investigations on diverse type of insects may give better insight to our existing knowledge. With this view, it was resolved to make certain investigations on *Utetheisa pulchella* larva which is phytophagous but preferably feeds on sannhemp plant in the northern India and it has hardly any alternative host for its survival in the field. The present paper deals with the observations on the digestive enzymes, their pH optima and distribution of some of the enzymes in the digestive organs of the above mentioned species.

Materials and Methods

In the laboratory a stock culture of *Utetheisa pulchella* was maintained in an incubator controlled at 30 °C $\pm$ 1° and approximately 80% relative humidity. The larvae were fed on green sannhemp leaves throughout the year. For the experiments described herein only the fully grown larvae of 5th instar were utilized.

(a) Preparation of extracts: Live larvae were dissected in Ringer's solution and their digestive tracts were taken out, pooled down and homogenised together to make concentrated extracts either in distilled water or known buffer solution according to the nature of the experiments. The strength of the extracts varied with different enzyme determinations (Table 1).

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Table 1

Digestive enzymes studied in the homogenates of complete digestive tract of *Utetheisa pulchella* LINNAEUS

Enzyme	Concentration of the extract	Substrate	Incubation time at 37 – 38°	Result
1. Pepsin-like	10 guts in 3 ml. of water	Sulphanitic acid azo-albumin	18 hours	–
2. Cathepsin-like	10 guts in 3 ml. of water	Sulphanitic acid azo-albumin	18 hours	–
3. Trypsin-like	10 guts in 3 ml. of water	Sulphanitic acid azo-albumin	18 hours	+
4. Polypeptidase	10 guts in 1 ml. of water	2% Peptone solution	1 hour	+
5. Dipeptidase	10 guts in 2 ml. of phosphate buffer (pH 8.0)	5% L-alanylglycine or 5% L-leucylglycine	3 hours	+
6. Invertase	10 guts in 3 ml. of water	5% sucrose solution	1 hour	+
7. Amylase	10 guts in 3 ml. of water	1.5% starch solution	1 hour	+
8. Maltase	10 guts in 1 ml. of water	2% Maltose solution	1 hour	+
9. Esterase	10 guts in 1 ml. of water	Emulsion of ethyl-butyrate	1 hour	+
10. Lipase	10 guts in 1 ml. of water	Olive oil Emulsion	1 hour	–
11. Acid phosphatase	25 guts in 2.4 ml. of water	0.5% sodium β'-glycerophosphate	1 hour	–
12. Alkaline phosphatase	25 guts in 2.4 ml. of water	0.5% sodium β'-glycerophosphate	1 hour	+

(+) Indicates positive enzyme activity
(–) Indicates no enzyme activity

(b) Determination of enzyme activity: For the determination of Peptic-, Cathepsin-, and the tryptic activity in the respective homogenates the technique was quantitative and based on the colorimetric method of TOMARELLI et al (1949), using sulphanilic acid azo-albumin as substrate.

The investase and amylase activity were also determined quantitatively by colorimetric method which involves the use of dinitro-salicylic acid reagent to develop the colour with the reducing sugar (SUMNER, 1925). The respective enzyme activity was measured in terms of the scale divisions of Klett Summerson photoelectric colorimeter by comparing the colour of the final solutions in the samples of each enzyme activity with the proper reagent control which was treated with like other samples except that it did not receive the extract. Besides, for each enzyme activity controlled samples in which the enzymes were destroyed by heating were also used for comparison. The activity of polypeptidase, maltase, esterase and lipase was determined only qualitatively. The mixtures of all the enzyme extracts and their respective substrates were incubated at 37 °C for different periods (Table 1).

(c) Determination of pH optima of enzymes: The pH optima of Trypsin-like enzyme, invertase and amylase were determined quantitatively. In each case samples of similar quantity were used from a concentrated extract prepared in distilled water. These samples were then separately mixed with equal quantity of different buffer solutions. The rest of the procedure was the same as described above.

(d) Distribution of invertase, amylase and Trypsin-like enzymes in the digestive organs: The distribution of these enzymes was studied in the extracts of whole foregut, midgut and hindgut separately and also in those of their respective tissue. In each case concentrated extract was made from 10 larvae in 1.0 ml. of buffer adjusted to the optimum pH for each enzyme. Similarly the extracts of 10 pairs of complete salivary glands with their contents were also made for each enzyme. To obtain a comparative data equal volume of samples were used for the determination of different enzyme activity.

Results

The data in table 1 shows the result of the determination of certain digestive enzymes of *Utetheisa pulchella* larva in the concentrated homogenates of com-

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plete digestive tracts. In this species as regards the occurrence of the proteolytic enzymes comparable to pepsin, trypsin and cathepsin of vertebrates, only a trypsin-like enzyme has been detected, which works between pH range 5.0 to 10.0 and shows its maximal activity at pH 8.0 (Fig. 1). The invertase and amylase are significantly present and their working range is between pH 5.0 to 8.0 and pH 5.0 to 10.0 respectively (Fig. 2). The invertase has its maximal activity

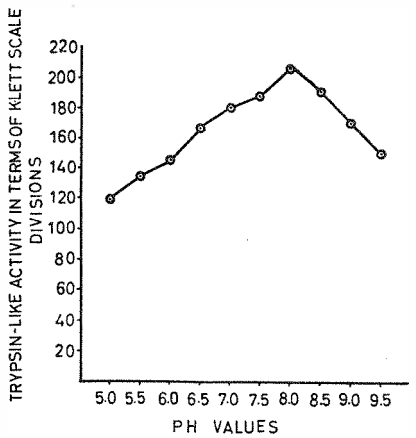

Fig. 1. Curve showing the trypsin-like activity at different pH values in the digestive tract extract of *Utetheisa pulchella* LINNAEUS larva

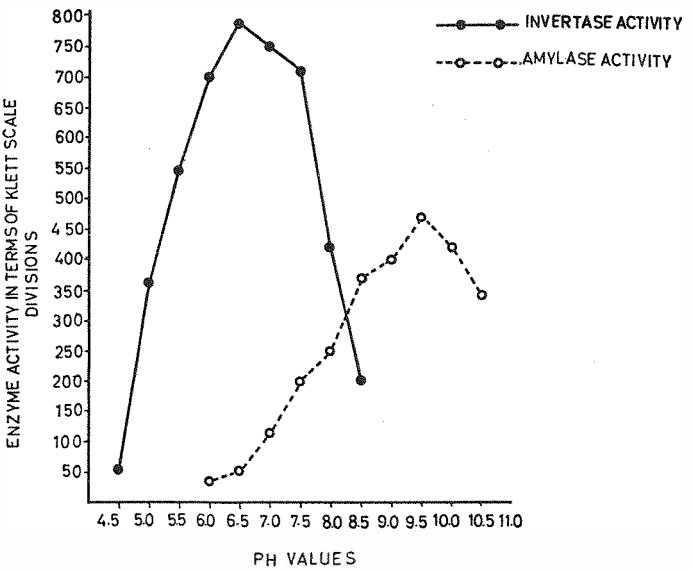

Fig. 2. Curves showing the invertase and amylase activity at different pH values in the digestive tract extract of *Utetheisa pulchella* LINNAEUS larva

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at pH 6.5 whereas that of amylase occurs at pH 9.5. A qualitative determination of maltase, esterase, lipase, polypeptidases, dipeptidases and alkaline phosphatase as well as acid phosphatase was also carried out. But, however, the activity of lipase and acid phosphatase could not be detected. Dipeptidases capable of hydrolysing L-alanylglycine and L-leucylglycine are fairly distributed in the midgut tissue, as well as in the contents of the midgut lumen. A detailed account on the distribution of these enzymes will be published elsewhere. The alkaline phosphatase activity is also significant in the larva from the development point of view, which has been discussed earlier (KHATOON, 1964). Thus in the larva of *U. pulchella*, the digestive juice contains the important enzymes capable of hydrolysing the major constituents of its natural food.

In a preliminary experiment it was found that the separate extract of fore-, mid- and hindgut the activity of invertase, amylase and trypsinlike enzyme occurred only in the midgut extract. This suggested to investigate the site of production of these enzymes in the digestive tract, as well as, the salivary glands. Therefore, the activity of these enzymes was determined in only the tissue extracts of fore-, mid- and hindgut separately. Since, separation of the tissue of the salivary gland was not possible, therefore, whole salivary glands were homogenised. It showed that in the alimentary canal all the three enzymes mentioned above are located only in the midgut tissue, however, the extracts of the salivary glands also indicated the presence of these enzymes. It is further evident that in the total salivary glands the invertase is higher in concentration than in the midgut tissue at the time of enzyme determination (Table 2).

Table 2

Distribution of invertase, amylase and trypsin-like enzyme in the salivary glands and in the tissue of regions of digestive tract of *Utetheisa pulchella* LINNAEUS (ten larvae were used in each case)

Enzyme	Enzyme activity in terms of Klett Units			
	Foregut	Midgut	Hindgut	Salivary glands
1. Trypsin-like	—	52 ku	—	35 ku
2. Invertase	—	105 ku	—	260 ku
3. Amylase	—	200 ku	—	32 ku

But this data is, however, not comparable because the enzyme from the midgut tissue may be continuously washed into the lumen of the midgut even in unfed condition. Further investigation showed the highest concentration of invertase and amylase in the anterior most region of the midgut tissue (Table 3).

Invertase is almost negligible in the posterior midgut whereas amylase is secreted as significantly as in the middle part of the midgut tissue. The trypsin-like enzyme shows almost equal distribution in all the regions.

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Table 3
Distribution of invertase, amylase and trypsin-like enzyme activity in the midgut tissue of *Utetheisa pulchella* LINNAEUS (ten larvae were used in each case)

Enzyme	Enzyme activity in terms of Klett Units		
	Anterior midgut	Middle midgut	Posterior midgut
1. Trypsin-like	13.0	12.0	14.0
2. Invertase	50.0	20.0	0.0
3. Amylase	116.0	53.0	51.0

Discussion

The pH optima for proteinases in the digestive juice of insects vary in different insects studied so far and sometimes the variation occurs on different substrates. But, generally proteinase of most of the insects are trypsin-like enzyme which has mostly its pH optimum in fairly alkaline range (WIGGLESWORTH, 1953; DAY & WATERHOUSE, 1953; CHAMPLAIN & FISK, 1956). But, however, in *Bombyx mori* (SHINODA, 1930) this enzyme shows its maximal hydrolysis of Caesin and gelatin at pH 11.5 and 9.2 respectively which are highly alkaline conditions. In contrast although both *B. mori* and *U. pulchella* are phytophagous lepidoptera, in the latter species the digestive juice hydrolyses a red protein (sulphanilic acid azo-albumin) even in acid range but having its maximal activity in fairly alkaline medium and showing less activity in the highly alkaline range.

In *U. pulchella* the extracts of the digestive tract shows the activity of the carbohydrases both in acid and alkaline range. But the maximal activity of invertase lies in very weakly acid condition which is almost similar to other phytophagous and omnivorous insects (UVAROV, 1928 and 1948; WIGGLESWORTH, 1927; DAY & POWNING, 1949; KRISHNA, 1958) and also that of the parasitic larvae of horse botfly, *Gastrophilus intestinalis* (TATCHELL, 1958). On the other hand, the maximal activity of amylase occurs in the highly alkaline range, i.e., pH 9.5 which is similar to that of *B. Mori* and also to that of a nematode parasite *Ascaris lumbricoides* (ROGERS, 1940). It can, therefore, be suggested that in the digestive tract of *U. pulchella* digestion of sugar and starch may be slow and occur simultaneously when the pH of the alimentary canal remains from weakly acid to weakly alkaline condition. But, however, very active and rapid digestion of either of these carbohydrates may occur only at different times when suitable pH conditions for the respective enzymes may become prevalent in the digestive tract. Such changing conditions of the gut are very possible after the intake of food and during the process of its digestion in the midgut. As regards the digestion of protein it can occur actively and simultaneously when the alkaline condition for the activity of amylase is prevalent.

The localization of the activity of invertase, amylase and trypsin-like enzyme in the midgut tissue suggests the secretory function of the midgut. The invertase

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is secreted only in the anterior and middle part of the midgut suggesting that the posterior part of the midgut tissue perhaps absorbs the digested sugars. Whereas amylase is secreted from the posterior midgut as well and hence this would suggest that the anterior part of the midgut is a very active site for the secretion of both invertase and amylase, and there by the digestion of sugar and starch can mainly occur in the anterior region of the midgut. But the secretion of a trypsin-like enzyme is fairly distributed over the entire length of the midgut epithelium suggesting that the digestion of protein may occur almost to the same degree in any region of the midgut. This would mean that the idea of 'ferment chain' for the digestion of protein as explained by SCHLOTTKE (1937) in Periplaneta is not supported by the present observation in the larva of *U. pulchella*. This proposition has been discussed in detail elsewhere (Khatoon, in press). The respective enzyme activity present in the tissue of the midgut is much less than the activity of these enzymes in the extract of the whole midgut including its lumen contents. This is pointing to the fact that the epithelial tissue of such an insect like *Tenebrio molitor* (DADD, 1956) and *Locusta migratoria* (KHAN, 1963), in phytophagous larva of *Utetheisa pulchella* as well the enzymes synthesized in the tissue are washed away into the midgut lumen continuously and most likely correlated with the continuous mode of feeding and digestion.

Although in *U. pulchella* the extra-intestinal digestion of food may not be ruled out the invertase, amylase and trypsin-like enzyme of the saliva may also contribute to intestinal digestion. However, foregut of the larva of *U. pulchella* being very short serves only a purpose of a passage from the mouth to the midgut because there is no secretion in the tissue of the foregut and also the food does not accumulate in this region for considerable time for the action of the enzyme. Moreover, since the extract of the foregut including its lumen contents does not show the activity of these enzymes, therefore, there is no possibility of digestion of protein, starch and sugar in this region. It is, therefore, obvious that invertase, amylase and a trypsin-like enzyme of the saliva are transported to the midgut along with the food and these enzymes become active to take part in digestion in the optimal pH conditions occuring in the midgut lumen.

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Summary

Some observations on the secretion of digestive enzymes were made in the fully grown larva of *Utetheisa pulchella* LINNAEUS. 1. The extracts of the digestive tract show the presence of trypsin, polypeptidases, dipeptidases, alkaline phosphatase, invertase, amylase, maltase and esterases. — 2. The salivary gland extracts have invertase, amylase and trypsin-like enzymes. — 3. The maximal activity of trypsin, invertase and amylase occurs at pH 8.0, 6.5 and 9.5 respectively. — 4. The secretion of invertase and amylase is largely localized in the anterior third of the midgut tissue, but some amylase is also secret-

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ed from the tissue of the posterior midgut. — 5. Trypsin-like enzyme is almost uniformly secreted from the tissue of the whole midgut. — 6. These enzymes are not present in the tissue of fore- and hindgut.

Zusammenfassung

Es wurden einige Untersuchungen über die Sekretion von Verdauungsenzymen bei der voll ausgewachsenen Larve von *Utetheisa pulchella* LINNAEUS angestellt. 1. Die Extrakte aus dem Verdauungstrakt wiesen Trypsin, Polypeptidasen, Dipeptidasen, alkalische Phosphatase, Invertase, Amylase, Maltase und Esterasen auf. 2. Die Extrakte aus der Speicheldrüse zeigten Invertase, Amylase und trypsinartige Enzyme. 3. Die größte Aktivität von Trypsin war bei pH 8,0, von Invertase bei pH 6,5 und von Amylase bei pH 9,5 zu verzeichnen. 4. Die Sekretion von Invertase und Amylase findet größtenteils im vorderen Drittel des Mitteldarmgewebes statt, doch wird auch etwas Amalyse vom Gewebe des hinteren Mitteldarms ausgeschieden. 5. Das trypsinartige Enzym wird fast einheitlich vom Gewebe des ganzen Mitteldarms ausgeschieden. 6. Diese Enzyme sind im Gewebe des vorderen und hinteren Teils des Verdauungskanals nicht vorhanden.

Резюме

Делались несколько исследования секреции энцимов пищеварения у взрослой личинки *Utetheisa pulchella* LINNAEUS. 1. Экстракты из желудочно-кишечного тракта содержали трипсин, полипептидазы, дипептидазы, щелочную фосфатазу, инвертазу, амилазу, малтазу и эстеразы. 2. Экстракт из слюнной железы содержал инвертазу, амилазу и энцимы, похожие на трипсин. 3. Высшая активация трипсина лежала у pH 8,0, у инвертазы у pH 6,5 и у амилзаы у pH 9,5. 4. Секреция инвертазы и амилазы осуществляется в большинстве в первой (1/3) части тканей средней кишки, немножко амилазы выделяется и в последних частей. 5. Энцим, похожый на трипсин, выделяется везде в тканях средней кишки. 6. Этот энцим отсутствует в передней и задней части желудочно-кишечного аппарата.

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