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Digestive physiology and food utilization of the larvae of *Earias vittella* (Lepidoptera: Noctuidae) from its malvaceous host plant

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Abstract. The hydrogen-ion concentration of the contents of fore-, mid- and hindgut of the larva of *Earias vittella* (F.) lays within a pH range of 8·8 and 9·6. These caterpillars possessed the ability to break down starch, raffinose, maltose, melibiose, sucrose and proteins. Synthesis of certain higher oligosaccharides indicating transglycosidic activity was also noticed during hydrolysis of raffinose, melibiose and sucrose in *in vitro* experiments. The activity of carbohydrases detected in the larval midgut was variably influenced by the hydrogen-ion concentration of the medium. Further, they differed from one another in their relative strength. Amylase was the most powerful and α-galactosidase was the weakest of all. An *in vivo* examination into the fate of fed starch, maltose, cellobiose and lactose within the gut revealed the physiological competency of the caterpillar to utilise only the first two carbohydrates (the former partly and the latter completely) through swiftly operating processes of digestion and absorption.

Movement of ingested food through the gut of the caterpillar was a rapid process. The first lot of food residue passed out of the hindgut within a mean time of 103 min after feeding. However, the entire alimentary canal got cleared of the ingested food only 36 h after a meal.

Keywords. *Earias vittella*; larva; gut physiology; food utilisation.

1. Introduction

The spotted bollworm, *Earias vittella* (F.) (=*Earias fabia* Stoll) is a major pest of cotton and okra in the tropics (Butani and Jotwani 1984). Physiological competency of this pest, in terms of hydrogen-ion concentration, distribution, nature and characteristics of digestive enzymes, rate of movement of ingested food and metabolic transformations of some fed sugars within the gut of the caterpillar (the main damaging stage in the life of this insect), to utilise the various dietary constituents obtained while feeding on its above mentioned natural host plants has already been reported (Krishna and Pandey 1974; Krishna and Tripathi 1986; Tripathi and Krishna 1988). A reiteration of some of these findings in this communication together with unpublished information (Tripathi 1985) concerning (i) the appearance of higher saccharides during the hydrolysis of certain sugars under *in vitro* conditions in the larval midgut and (ii) the *in vivo* fate of certain carbohydrates in the caterpillar was considered appropriate for a meaningful comprehension of insect-plant relations, from nutritional physiology point of view, between this pest and its food plants.

2. Materials and methods

Third instar (5 days old) caterpillars of *E. vittella* reared on tender seeds of okra (Vishwapremi and Krishna 1974) were used for all tests. Before using them in the

various experiments, these larvae were allowed access to feed only on moist filter paper for a period of at least 24 h, placed inside glass containers (35 × 100 mm) covered at the top by muslin fastened by elastic bands. Such pre-experimental treatment of the larva was found essential to clear its alimentary canal from all previously ingested food obtained from okra seeds.

2.1 *pH measurement of the gut*

The hydrogen-ion concentration of the different regions of the alimentary canal was determined by the 'range-indicator method' (Waterhouse 1940) after feeding the caterpillars for 10 min, on crushed okra seeds blended separately with 7 indicators (bromophenol blue, bromocresol green, bromocresol purple, bromothymol blue, phenol red, cresol red and thymol blue). A 20:1 ratio was maintained in the proportion of seed and dye components in all the mixtures. Such preparations also helped in finding out the pH range of okra seed (food of the larvae).

2.2 *Distribution, nature and characteristics of digestive enzymes*

This part of the study first involved *in vitro* monitoring of the presence of different digestive enzymes in the lumen of the caterpillar's midgut followed by determination of the influence of hydrogen-ion concentration on the activity of the detected carbohydrases and estimating their relative strength as well.

2.2a *Qualitative survey of digestive enzymes*: For an examination of this aspect, the caterpillars were first fed on glucose mixed with bromophenol blue (sugar-dye proportion maintained as before) for 5 min (for the purpose of providing adequate stimulus for proper amount of enzyme secretion) and then removed from food. Contents of the lumen of the midgut from each of 10 such fed larvae were extracted following the procedure of Krishna (1955, 1958) and pooled together. Later, 0·1 ml aliquot of this extract was incubated at 37°C for 24 h with similar volumes of (i) a suitable substrate, (ii) one of 4 arbitrarily chosen buffers having pH values 5·8, 6·8, 7·2 (McIlvaine 1921) and 9 (Oser 1971) and, where necessary, (iii) an activator. A drop of toluene was added to this mixture to prevent growth of microorganisms. The enzymes tested for and substrates employed are listed in table 1. After incubation, in case of carbohydrases, products of digestion and/or the presence of undigested substrate, if any, in the mixtures at different pH values were detected using appropriate biochemical tests or by adopting the technique of paper chromatography (Krishna 1958). Assessment of proteinase activity was based on the methodology initially implemented by Saxena and Bhatnagar (1958) and subsequently carried out by Krishna and Saxena (1962). Activities of polypeptidase and lipase were determined as described by Krishna (1955). Each enzyme experiment was repeated thrice and identical results were obtained. All trials were accompanied by properly set controls wherein the same quantity of distilled water replaced midgut extracts in each incubated mixture.

An interesting feature observed during the determination of activity of carbohydrases hydrolysing melibiose, raffinose and sucrose was the appearance, in every assay, on the chromatogram of a new sugar (in addition to the normal products of digestion of these carbohydrates), the R_g value (ratio of distance

Table 1. List of enzymes tested for and the substrates employed.

Enzyme	Substrate
Carbohydrases	
Amylase	Soluble starch, 0·3% solution
Invertase	Sucrose, 5% solution
Maltase (α-glucosidase)	Maltose, 5% solution
α-fructofuranase	Raffinose, 5% solution
α-galactosidase	Melibiose, 5% solution
β-glucosidase	Cellobiose, 5% solution
β-galactosidase	Lactose, 5% solution
Proteinase	Gelatin on photographic plate
Polypeptidase	Peptone, 5% solution
Lipase	Olive oil emulsion

migrated by the new sugar spot to that migrated by glucose spot on the chromatogram) of which showed great variation amongst them. It was of interest to ascertain the time of appearance and relative concentration (based on visual comparison of colour intensity of the spots on the chromatogram) of these unidentified sugars in the reaction mixtures kept for incubation at 37°C with one or the other of the 3 above mentioned carbohydrates. For this purpose, a separate series of experiments was arranged along the lines similar to that already described with the difference that the incubation mixture set up in all these tests was always maintained at pH 9. At the end of every hour of incubation up to a period of 7 h 30 μl sample was drawn from each reaction mixture and the sugars present in it, including the new saccharide, were resolved by paper chromatography as reported earlier.

2.2b *Influence of hydrogen-ion concentration on the activity of carbohydrases*: This part of the investigation first entailed the constitution of a series consisting of 6 samples of the midgut extracts of the caterpillar, prepared as outlined already. For determination of amylolytic activity, the volume of extract maintained in each sample was 0·2 ml. The reaction of the extract in these samples was adjusted to 6 different values of hydrogen-ion concentration by adding to each 0·2 ml of a suitable buffer formulated as mentioned earlier and ranging from 5·8–9·5. A control series comprising two samples, wherein an identical volume of distilled water was kept in place of gut extract, was also set up. One of these samples was similarly buffered to pH 5·8 while in the other the pH was regulated to 9·5. After adding 0·1 ml of 1% sodium chloride solution (serving as an activator) followed by 0·2 ml of 0·3% starch solution and two drops of toluene to each sample of both series, the mixtures were incubated at 37°C for 18 h. The reaction was then stopped by the addition of 0·15 ml of N-hydrochloric acid to each mixture. Each sample was then treated with 0·075 ml of potassium iodide-iodine reagent (Smith and Roe 1949) and the solution in it was finally diluted with distilled water to 5 ml.

Blue colour would develop in solutions, the intensity of which would depend upon the concentration of undigested starch in the different incubated mixtures. This concentration was measured colorimetrically (Smith and Roe 1949) using an

appropriate standard for eventual determination of per cent hydrolysis of starch at different pH values.

To estimate the activity of remaining carbohydrases, the volume of midgut extracts held in each of the 6 samples of the series was 0·1 ml. Controls were arranged as before to form a separate series. pH conditions in the samples of both these series were variously maintained, like in amylase experiment, by the addition of 0·1 ml of one of the prepared buffers. Solutions in each of these samples subsequently received 0·1 ml of a concerned substrate (5% solutions of maltose, melibiose, raffinose or sucrose) and two drops of toluene prior to keeping them for incubation at 37°C for 18 h. The rest of the methodology relating to paper chromatography, elution and colorimetry adopted for quantitative evaluation of the pH effect on each carbohydrase activity was basically akin to that described by Krishna (1958).

2.2c *Relative strength of different carbohydrases*: Consideration of this issue involved division of the pooled larval midgut extracts into 5 lots of 0·1 ml each. Every lot was then supplemented with 0·1 ml of a buffer of pH 9 [rationale for the choice of such pH value was to approximate the hydrogen-ion concentration of the experimental medium to that which naturally prevailed within the midgut of the caterpillar, as observed by Krishna and Tripathi (1986) and reported in this communication as well], 0·1 ml of an appropriate carbohydrate substrate of known concentration (0·3% in the case of starch and 5% with respect to other 4 sugars listed in the previous section) and, in tests relating to assessment of the strength of amylase activity, 0·1 ml of 1% sodium chloride solution to function as an enzyme activator. Two drops of toluene were then added to each sample and the mixtures were incubated at 37°C for 24 h. At the end of incubation, the reaction mixture containing starch was treated with 0·15 ml of N-hydrochloric acid to inhibit enzymatic activity of amylase. The follow-up procedure to determine the strength of amylase in the larval midgut, based on colorimetric estimation of hydrolysis of starch, was similar to that already described.

In the case of other incubated mixtures, the termination of incubation first led to the drawing out, from every sample, of two fractions of 0·02 ml each for eventual subjection to paper chromatography (Krishna 1958). The loci on the base line of chromatogram, where these fractions were applied, had been earlier treated with 0·007 ml of 0·1 N sodium hydroxide solution. This would ensure stopping of enzymatic reaction as soon as the incubated mixture was applied to the chromatogram. Concentration of the undigested substrate in each incubated mixture was subsequently determined by elution and colorimetry (Krishna 1958). Comparison of the degree of hydrolysis of the different substrates would indicate the relative strength of the carbohydrases.

2.3 *Passage of food through the gut*

For monitoring the movement of ingested food through the larval digestive tract, an individual was initially fed for 2 min on okra seed diet mixed with bromophenol blue. Dissections of such fed larvae were performed at different time intervals after the meal so as to determine the position of the dye in the various parts of the gut. This technique would apparently indicate the locus of ingested food in relation to a point of time, provided the dye behaves as a marker and its transport through the

larval gut synchronises with that of the food mixed with it. Time taken for the arrival of first coloured excreta after food intake was also recorded. For this purpose, each fed caterpillar, freed from adhering food particles, was transferred to a clean rectangular glass container (45 × 45 × 15 mm) having a glass top through which the insect was constantly observed for ejection of its first coloured faeces. Difference between the time of ingestion of the last meal and the evacuation of the first coloured excreta would indicate the rate of passage of the first lot of eaten food through the gut. The total time required for all the ingested food to pass through the alimentary canal of the caterpillar was also calculated in a similar manner. The entire course of experiment was repeated at least 5 times employing a fresh larva for each replicate.

2.4 *In vivo fate of certain carbohydrates*

In order to ascertain the time and site of digestion and absorption of certain individual nutrients *in vivo*, the fate of starch, maltose, cellobiose and lactose was followed along the lines as reported in a similar study involving fructose, sucrose and raffinose in this species (Krishna and Pandey 1974). However, in the present series of tests, the caterpillars were allowed to feed for 30 min on one of these carbohydrates mixed with bromophenol blue as per proportions already stated and samples of haemolymph and midgut contents from these fed larvae were collected at 30, 60, 90 and 120 min after a meal while that of excreta 6–8 h after nutrient intake for chromatographic analysis.

3. Results

3.1 *Hydrogen-ion concentration*

The hydrogen-ion concentration of all the 3 regions of the gut of *E. vittella* larva was moderately alkaline and lay within a range of pH 8·8–9·6 (table 2) despite the caterpillar feeding on okra seeds having a strong acidic property (pH 4·6–5).

3.2 *Digestive enzymes*

The presence or absence of various digestive enzymes, based on an *in vitro* investigation and determined qualitatively at 4 randomly chosen pH values, in the

Table 2. pH in different regions of the digestive tract of *E. vittella* larva.

Indicator	Foregut	Midgut	Hindgut
Bromophenol blue	>4·6	>4·6	>4·6
Bromocresol green	>5·2	>5·2	>5·2
Bromocresol purple	>6·8	>6·8	>6·8
Bromothymol blue	>7·6	>7·6	>7·6
Phenol red	>8·4	>8·4	>8·4
Cresol red	>8·8	>8·8	>8·8
Thymol blue	>8·4<9·6	>8·4<9·6	>8·4<9·6
Range of pH	>8·8<9·6	>8·8<9·6	>8·8<9·6

larval midgut of this noctuid species is given in table 3. Only 5 carbohydrases (amylase, invertase, maltase (=α-glucosidase), α-fructofuranase and α-galactosidase) and a proteinase were found to occur in the midgut of this caterpillar. Interestingly, the activity of α-galactosidase, α-fructofuranase and invertase, in addition to causing hydrolysis of their corresponding substrates, also led to the production of variable types of oligosaccharides of unknown composition (table 4). Although all these unidentified sugars made their appearance within the first hour itself of digestion of melibiose, raffinose and sucrose respectively, their relative concentration, however, varied with the passage of time during the breakdown of these substrates.

3.2a *Influence of hydrogen-ion concentration on the activity of carbohydrases*: The degree of activity of the various carbohydrases occurring within the midgut of the larva of *E. vittella* varied with the hydrogen-ion concentration of the medium (table 5). Amylase activity was very high exceeding 90% hydrolysis of starch at all tested pH values save 9·5 and reached a maximum at pH 7 or 8. Amongst the remaining carbohydrases, only invertase showed consistently more than 50% activity at different pH values peaking at pH 8 which was slightly higher than that recorded at pH 9. Maltase and α-galactosidase exhibited a similarity in having their highest level of activity at pH 6·4 while α-fructofuranase attained its zenith in bringing about hydrolysis only at pH 9.

3.2b *Relative strength of digestive carbohydrases*: The foregoing account clearly reveals that under the influence of any one common pH value different carbohydrases detected from the midgut of the larva of *E. vittella* possess varying capacities to hydrolyse their corresponding substrates. This indicates the existence of an obvious disparity in the relative strength of these carbohydrases. This also implies that greater the strength of a particular enzyme, the more will be the efficiency of the insect to digest the corresponding substrate. In other words, the degree of utilisation of different carbohydrates by this larva depends, to some extent, on the relative strength of the appropriate enzyme. With a view to check this

Table 3. Distribution of digestive enzymes in the midgut of the larva of *E. vittella* (*in vitro* study).

	pH values			
Enzyme	5·8	6·8	7·2	9·0
Carbohydrases				
Amylase	+	+	+	+
Maltase (=α-glucosidase)	+	+	+	+
Invertase	+	+	+	+
α-fructofuranase	+	+	+	+
α-galactosidase	+	+	+	+
β-galactosidase	−	−	−	−
β-glucosidase	−	−	−	−
Proteinase	+	+	+	+
Polypeptidase	−	−	−	−
Lipase	−	−	−	−

+, Indicates presence of enzyme; −, indicates absence of enzyme.

Table 4. Relative concentration of oligosaccharides synthesised, their mean R_g value (ratio of distance migrated by an oligosaccharide spot to that migrated by glucose spot on the chromatogram) and break-up of other hydrolytic products detected during the course of activity, within a stipulated time frame, of α-galactosidase, α-fructofuranase and invertase at pH 9 from the midgut of *E. vittella* larva (*in vitro* study).

Enzyme (substrate)	Duration (h) of hydrolysis of the substrate, products detected and relative concentration (denoted by increasing number of plus signs) of the oligosaccharide (O) formed							Mean R_g value* of oligosaccharide
	1	2	3	4	5	6	7	
α-galactosidase								
(melibiose)	mb ga g O (+)	mb ga g O (+ +)	mb ga g O (+ +)	mb ga g O (+ + +)	mb ga g O (+ + +)	mb ga g O (+ +)	mb ga g O (+)	0·07
α-fructofuranase								
(raffinose)	r mb ga g f O (+)	r mb ga g f O (+)	r mb ga g f O (+ +)	r mb ga g f O (+ + +)	r mb ga g f O (+ +)	r mb ga g f O (+)	r mb ga g f O (+)	0·18
Invertase								
(sucrose)	s f g O (+ +)	s f g O (+ +)	s f g O (+ + +)	s f g O (+ + +)	s f g O (+ +)	s f g O (+)	s f g O (+)	0·38

*Based on 8 determinations.

f, Fructose; g, glucose; ga, galactose; mb, melibiose; r, raffinose; s, sucrose.

Table 5. Estimates of per cent hydrolysis of various carbohydrates at different selected pH values by corresponding carbohydrases from the midgut of *E. vittella* larva.

Carbohydrase	Substrate	Per cent hydrolysis (rounded to the nearest integer) of substrate at different selected pH values					
		5·8	6·4	7·0	8·0	9·0	9·5
Amylase	Starch	92	93	97	97	93	72
Invertase	Sucrose	51	67	52	70	69	61
Maltase (=α-glucosidase)	Maltose	22	62	58	51	30	0
α-fructofuranase	Raffinose	15	18	26	22	40	20
α-galactosidase	Melibiose	24	27	17	12	11	0

point and to obtain a clear confirmation of this phenomenon in the caterpillar's digestive physiology, the activities of 5 carbohydrases described above have been quantitatively assayed at one common pH value over an extended period of experimental time.

Amongst the different carbohydrases, amylase was the strongest since almost the entire substrate (99·2%) was hydrolysed. Next in order came invertase and α-fructofuranase which hydrolysed 72·5 and 50·5% respectively of their corresponding substrates. Maltase was a moderately active enzyme and degraded 40·5% of the disaccharide maltose while α-galactosidase was the weakest of the carbohydrases capable of breaking down only 15% of melibiose to its monosaccharide components.

3.3 *Movement of food through the gut*

The ingested food passed quite rapidly through the larval foregut and accumulated in its midgut. Within 6–10 min of feeding, the first lot of food residue began to enter into the hindgut where it stayed for a period of 70–122 min before its eventual defaecation. In the midgut, food, nonetheless, continued to remain even beyond 16 h. However, by the end of 24 h, there was hardly any food noticed in this region. The entire alimentary canal of the caterpillar, however, got cleared of all the ingested diet within a mean time of 36 h after a meal.

3.4 *In vivo analysis of certain carbohydrates*

Although observations procured from *in vitro* analysis of the distribution of various carbohydrases in the midgut of the larva of *E. vittella* described above, suggest the ability of this insect to utilise the corresponding substrates, they do not provide clue concerning the actual time of digestion of all these nutrients and subsequent absorption of products yielded from such digestion within the caterpillar's midgut. In order to acquire more information on this aspect, the fate of starch, maltose, cellobiose and lactose ingested by this insect has been traced.

Table 6 summarises the findings relating to the occurrence of one or the other of the aforementioned ingested carbohydrates in the midgut, haemolymph and excreta of the caterpillar and their subsequent biochemical transformations within the former two tissues of the insect body. Within 30 min of a meal, all the 4 eaten

Table 6. Carbohydrates in the midgut (M), haemolymph (H) and excreta of *E. vittella* larva at different time intervals after ingestion of starch, maltose, cellobiose or lactose (*in vivo* study).

	Time interval (min)								
	30		60		90		120		Excreta (collected 6–8 h after feeding)
Carbohydrate	H	M	H	M	H	M	H	M	
Starch		st		st		st		st	st
		m	m	m	m	m	m	m	
	g	g	g	g	g	g	g	g	
Maltose		m	m	m	m	m	m	m	
	g	g	g	g	g	g	g	g	
Cellobiose		c		c		c		c	c
Lactose		l		l		l		l	l

c, Cellobiose; g, glucose; l, lactose; m, maltose; st, starch.

saccharides were detected in the midgut, although starch and maltose during this period had undergone partial hydrolysis to yield small amounts of products of their digestion. At the same time, some glucose began to show up in the haemolymph of caterpillars fed on either of these two carbohydrates. As the time interval after ingestion of starch or maltose increased to 60 min, the latter sugar also, interestingly, started to appear in the larval haemolymph. Neither cellobiose nor lactose could be digested by this caterpillar in its ventricular region at any time during the entire 2 h experimental period. Chromatographic analysis of the larval excreta collected even as late as 6–8 h after the insect feeding on each of these two carbohydrates further showed that the food residue contained cellobiose and lactose. Curiously enough, some undigested starch was also detected in such faecal matter.

4. Discussion

This investigation highlights several interesting features in the digestive physiology of *E. vittella* larva which warrant elucidation in the light of whatever information is available on similar issues on other phytophagous insects, more importantly with reference to different species of noctuids.

The high pH values having identical range in all the 3 regions of the gut of this caterpillar are quite at variance with those reported earlier for the same insect (Srivastava and Mathur 1966). The adoption of indicator paper touch method by these authors, where contamination of the paper by the surrounding haemolymph was an inevitable drawback, had presumably seriously affected the proper determinations of the hydrogen-ion concentration of this insect's digestive system in their studies. It must also be mentioned here that the enhanced pH value recorded in the midgut in this study fits in with the generally prevalent more alkaline nature of the contents of the gut in phytophagous insects (Wigglesworth 1984). The absence of any change in the gut pH, despite the caterpillar feeding on okra seeds possessing strong acidic nature, evidently shows the existence of a powerful buffering mechanism within the larval gut as in a majority of other insects (House 1974).

As in the midgut of caterpillars of two other noctuids, *Spodoptera litura* and *Trichoplusia ni* (Mathur 1966), *E. vittella* larva also exhibited the activity of amylase, invertase, maltase and a proteinase in its ventricular region and thus possessed the capacity to digest starch, sucrose, maltose and proteins. However, the spotted bollworm differed from *S. litura* and *T. ni* in not being endowed with the ability to hydrolyse lactose or lipids in view of the absence of β-galactosidase and lipase. The competency of this caterpillar to digest melibiose observed in the present investigation contradicts the earlier report of Krishna and Pandey (1974) on the non-existence of α-galactosidase enzyme in this larva. One possible reason for the failure of these authors to detect the presence of this enzyme (the weakest of 5 carbohydrases recorded in the larval midgut) is their adoption of an indirect method of testing for the occurrence of this enzyme through raffinose hydrolysis (instead of using melibiose, a more appropriate substrate). The presence of cellobiose and lactose in the excreta discharged by caterpillars fed on these sugars coupled with the absence of β-glucosidase and β-galactosidase in the larval midgut (as noticed in *in vitro* tests) confirms the total inefficiency of this insect to utilize

these sugars, both of which do not occur in the tender seeds of okra or cotton (natural host plants of this insect) (Mehta and Saxena 1973; Mani *et al* 1980). The appearance of some starch, despite its hydrolysis within the insect's midgut by a very powerful amylase, in the faeces of caterpillars ingesting this polysaccharide endorses the observations of Mehta and Saxena (1973) pointing to the existence of a limitation in the utilisation of this carbohydrate by these individuals. Presumably, amylase serves as a useful adjunct in the larval gut enzymes profile to provide the sugar requirements of the insect through partial digestion of starch, whenever obtained through feeding either from natural malvaceous food plant cotton or from certain experimentally tested non-malvaceous crops like *Zea mays* and *Pisum sativum* (Mehta and Saxena 1973) all of which, perhaps, contained some chemical ingredients restraining the activity of this carbohydrase *in vivo*. The occurrence of appreciably high levels of invertase and α-fructofuranase activity would obviously be of great value to the larva to properly utilise the sucrose present in its foods (Mehta and Saxena 1973; Mani *et al* 1980) and raffinose (determined only in *P. sativum*) (Mehta and Saxena 1973) eaten by it. Since maltose or melibiose, as such, was not present at all in the diets used in experimental studies with this insect (Mehta and Saxena 1973; Mani *et al* 1980), the enzyme maltase might be coming into action subsequent to the production of maltose derived from hydrolysis of starch by amylase and the role of α-galactosidase might be to breakdown melibiose following its yield from the digestion of raffinose by α-fructofuranase. This partly explains for the presence of these enzymes at very low strength. Correlation of the influence of hydrogen-ion concentration on the activity of different detected carbohydrases with pH range prevalent within the midgut of the larva of *E. vittella* clearly indicates that the lumen of this region in the alimentary canal of this caterpillar is most favourable only for the activity of invertase and α-fructofuranase although considerable digestion of starch is also possible.

Results on the study of fate of certain carbohydrates within the midgut of this larva clearly revealed that digestion of starch and maltose occurred during the first half hour after ingestion. Appearance of glucose at the same time in the larval haemolymph indicated a simultaneous absorption of the product of digestion of these two carbohydrates from the midgut. The absorption of some ingested maltose as such into the blood of the larva 1 h after a meal is, indeed, interesting and, in this respect, it not only resembled another disaccharide sucrose (Krishna and Pandey 1974) but also served as one more experimental evidence in support of the statement 'occurrence of sugars in insect haemolymph is also consistent with relatively non-specific absorption' (Wyatt 1967).

An important factor that affects digestion and absorption of food materials in insects, in general, is the period of stay of these substances in the digestive and absorptive portions of the gut of these animals. The longer the duration of halt of a food constituent in these areas, the greater are the chances of its complete digestion and subsequent absorption. In *E. vittella* larva, although the first lot of ingested food that diffused in the midgut took only a mean time of 7·7 min to enter the hindgut, the overall period of stay of the diet was considerably longer in the ventricular region and always extended beyond 16 h since intake of a meal. This was, undoubtedly, much greater than that reported for food movement in *S. litura* and *T. ni* (Mathur 1967). The prolonged duration of retention of food in the larval midgut of *E. vittella* coupled with the ability of this caterpillar to swiftly digest the

fed carbohydrates and absorb their hydrolytic products (Krishna and Pandey 1974 and present data) strongly suggest that these physiological characteristics of the gut of this insect are well established to ensure maximum utilisation of these carbohydrates.

The presence of a new unidentified oligosaccharide, besides appearance of hydrolytic products, in midgut in *in vitro* tests involving raffinose, melibiose or sucrose confirms transglycosidic activity, a biochemical phenomenon excellently manifested in many phytophagous insects (Waterhouse 1957; Saxena and Bhatnagar 1961; Saxena and Krishna 1963; Krishna and Pandey 1974; House 1974; Srivastava and Krishna 1977), of α-fructofuranase, α-galactosidase and invertase in spotted bollworm caterpillar. The fact that the unknown higher saccharides synthesised in the course of degradation of these sugars were all not the same (based on their R_g values) provides a reasonable basis to suggest the existence, in *E. vittella* larva, of varying patterns of transglycosidic action which, however, has a relationship with the substrate on which the carbohydrase reacts initially.

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