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# The Digestive Enzymes in the Alimentary System of *Acrotelsa collaris* (Thysanura: Lepismatidae)

by

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## INTRODUCTION

This paper deals with qualitative tests for detecting the enzymes present in the tissue and luminal contents of the alimentary system of *Acrotelsa collaris*. Since most or all of these enzymes would be available for digestion, a knowledge of them could give some indication of the feeding habits of the silverfish, and therefore of its adaptability to different living conditions.

Wall & Swift (1954) have detected maltase, invertase, lipase, trypsin, and " erepsin " in the gut of the lepismatid, *Thermobia domestica*, but not amylase, lactase or pepsin. No tests were made for $\beta$–glucosidase, $\alpha$–galactosidase, and cellulase.

Lasker & Giese (1956) found a cellulase in the midgut of bacteria-free *Ctenolepisma lineatum*, but not in the crop or salivary gland. Amylase and cellobiase were also present in the midgut.

## MATERIAL AND METHODS

Etherised specimens of *Acrotelsa collaris* were dissected in distilled water or glycerol, and the tissue of the alimentary system, with contents, was separated into four parts : (1) stomodaeum of head and prothorax, along with the salivary tissue and the general head tissue; (2) rest of the stomodaeum consisting mostly of the crop; (3) mesenteron; and (4) proctodaeum.

For each test the same gut regions from four or five specimens were broken up together in 0·05 ml. of distilled water or glycerol and ground in this extracting fluid, alone or with 0·10 ml. of the substrate when this was a fluid. Usually 0·05 ml. of a buffer solution, of a pH known to favour the particular enzyme action, was added to extract and substrate. The mixtures were then incubated in stoppered micro tubes at 36 –38°C for some days, thymol or toluene being used as preservative.

At the end of the period of incubation the presence of products of enzyme action, or the absence of substrate, in the mixtures, was tested for or observed directly.

Boiled glycerol extracts, for use in control mixtures, were made by placing in boiling water for ½ to 1½ hours.

Tests were repeated, sometimes more than once, when necessary.

*Carbohydrases*

The specimens used in testing for carbohydrases were confined for three weeks or over in beakers containing only particles of *maldive fish**. This high protein diet resulted in the absence of free glucose from the gut extracts, and therefore from the mixtures before incubation.

The gut regions were dissected out, broken up, and ground, in distilled water, since glycerol is known to inhibit the action of certain carbohydrases (Pavlovsky & Zarin, 1922; Wigglesworth, 1927). The appropriate substrate (see Table 1) was added before maceration. A crystal of thymol served as preservative, and the buffer solution used was Sorensen's phosphate buffer, pH 5·3, in all the tests except in the test for amylase, where the phosphate buffer, pH 6·5, was used.

TABLE 1

| *Carbohydrase* | *Substrate* |
|---|---|
| α-Glucosidases | |
| maltase | ½ % maltose |
| sucrase | 3 % sucrose |
| β-Glucosidase | 2 % salicin |
| β-Galactosidase | 3 % lactose |
| Amylase | 1 % soluble starch |
| Cellulase | Cellulose suspension (Trager, 1932). |

The mixtures were tested initially for glucose by the *Clinistix** Test (Varley, 1962); and then again after an incubation period of 4 – 5 days. In addition, in the test for amylase, a part of the mixtures after incubation was tested for starch with iodine solution.

Control mixtures containing (1) boiled gut extracts, (2) no substrate, and (3) no gut extracts, were also incubated under otherwise identical conditions, and tested before and after for glucose.

*Proteases*

(*a*) Trypsin-like Proteinase –(1) using casein substrate:

The gut regions were macerated in glycerol and 1% alkaline casein solution (Baldwin & Bell, 1955), and the mixtures incubated with Sorensen's phosphate buffer, pH 8·0, under toluene, for 9 days. The mixtures were then centrifuged, and the clear, supernatant liquid tested for casein with 1% acetic acid.

A control mixture without gut extract was also incubated and tested.

–(2) using fibrin substrate:

Glycerol extracts were incubated under toluene, with the buffer, pH 8·0, and with small pieces of fibrin coloured with congo red.

*This is salted, smoked and dried skipjack, ***Euthynnus pelamis***, mainly. A dried Japanese preparation of the same fish contains 75.6% protein, 5.1 % fat, 1.0% starch and sugar, and 14.3% water, according to a Government of Japan publication (1954), "Standard Table of Food Composition in Japan".

*Trade name; Ames & Co., London.

The accompanying control mixture contained no gut extract.

(*b*) Pepsin-like Proteinase –(1) using casein substrate:

The procedure was the same as for trypsin, except that an acid casein solution (Baldwin & Bell, 1955) with buffer solution, pH 2·6 (McIlvaine Lillie, citric acid –phosphate), or with no buffer at all, was used; and that the testing for casein after incubation was done with 10% sodium acetate solution.

Two control mixtures, one without gut extract and the other containing boiled extracts of crop and mesenteron together, were incubated and tested for casein.

–(2) using fibrin substrate:

The procedure was as for trypsin, except that the pH was adjusted to 2·6 with McIlvaine Lillie buffer. After 7 days' incubation a volume of 1 M disodium phosphate calculated to change the pH to 8·0, was introduced into each mixture.

(*c*) "Erepsin":

The gut regions were macerated in distilled water and 3% peptone solution. Incubations were made with Sorensen's phosphate buffer, pH 8·0, and under toluene. After 8 or 9 days the mixtures were centrifuged, and 5% acetic acid and bromine water added to the supernatant liquid. Shaking with butanol facilitated observation of any pink colouration due to tryptophan.

An accompanying control mixture contained no gut extract.

*Lipase*

The gut regions were macerated in glycerol and an olive oil emulsion containing the indicator phenol red (Baldwin & Bell, 1955). The mixtures, after addition of toluene were just turned pink with 2% sodium carbonate solution, and incubated.

Three control mixtures containing (1) no gut extract, (2) boiled crop extract, and (3) boiled mesenteron extract, were also incubated.

## RESULTS

The results obtained are summarised in Table 2.

TABLE 2

| | *Anterior stomodaeum Salivary tissue* | *Crop* | *Mesenteron* | *Proctodaeum* |
|---|---|---|---|---|
| Carbohydrases | + | + | + | + |
| Trypsin-like Proteinase | – | + | + | – |
| Pepsin-like Proteinase | – | + | + | – |
| "Erepsin" | – | + ? | + | + |
| Lipase | – | + | + | – |

*Carbohydrases*

Glucose was absent from the test mixtures and control mixtures before incubation, and from the control mixtures after incubation. However, the test mixtures after incubation contained glucose, showing the presence of all the carbohydrases for which tests were made.

In the tests with the cellulose substrate, the intensity of the *Clinistix* reaction after the incubation period was less than with the other carbohydrate substrates.

The test mixtures containing starch substrate initially, showed no trace of starch after incubation. The starch in the controls was present unchanged.

*Proteases*

(*a*) Trypsin-like Proteinase –(1) using casein substrate:

Acetic acid gave a white precipitate with the test mixtures containing anterior stomodaeum/salivary tissue and proctodaeum extracts, and with the control mixture. This shows the presence of unchanged casein after incubation. Casein had however disappeared from the test mixtures containing the crop and mesenteron extracts, for acetic acid gave no precipitate.

–(2) using fibrin substrate:

The pieces of fibrin in the mixtures containing anterior stomodaeum/salivary tissue and proctodaeum extracts, and in that without gut extracts, were not appreciably changed, even after 7 days. However after the same period, fragmentation of the fibrin by the crop and the mesenteron extracts was quite marked.

(*b*) Pepsin-like Proteinase –(1) using casein substrate:

The test mixtures containing anterior stomodaeum/salivary tissue and proctodaeum extracts, and the control mixtures, gave a white precipitate with sodium acetate. The test mixtures containing crop and mesenteron extracts gave no precipitate, indicating the disappearance of casein during incubation.

This test was done four times with the same result.

–(2) using fibrin substrate:

At pH 2·6 slight fragmentation and liberation of red colour were observed only in the mixture containing crop extract.

Three days after changing to pH 8·0, fragmentation and liberation of colour, showing trypsin activity, were most evident in the mixture containing mesenteron extract, and to a lesser extent in the mixtures containing anterior stomodaeum/salivary tissue and crop extracts. There was no fragmentation of fibrin by the proctodaeum extract.

(*c*) "Erepsin":

A pink colouration (+ sign), indicating the formation of free tryptophan, was given in the three tests made, in the way shown in Table 3.

The control mixtures gave no colouration.

TABLE 3

| | *Anterior stomodaeum Salivary tissue* | *Crop* | *Mesenteron* | *Proctodaeum* |
|---|---|---|---|---|
| Test 1 | − | − | + | + |
| Test 2 | − | − | + | + |
| Test 3 | − | + | − | + |

*Lipase*

Liberation of fatty acids, shown by a change in colour of the internal indicator from pink to yellow, took place in the mixtures containing extracts of crop and mesenteron The mixtures containing anterior stomodaeum/salivary tissue and proctodaeum extracts and the control mixtures, remained pink.

## DISCUSSION

The number and variety of enzymes in the gut of *Acrotelsa collaris* are indicative of an omnivorous condition, and the need for a particular type of diet is therefore not a factor likely to restrict the range of the silverfish. It seems to be able to utilise starch, cellulose, sugars, proteins and fats.

According to Wall & Swift (1954), "the nutritional essentials of domestic cockroaches and silverfish are closely allied" because of the similarity between the digestive enzymes of *Thermobia domestica* and of *Blatta orientalis* (Swingle, 1925). Also, the habits of *T. domestica* "are very much like those of the German cockroach" (Spencer, 1930). The similarity of *A. collaris* to the omnivorous cockroaches is as close; like the cockroaches (Wigglesworth, 1927) and unlike *T. domestica*, this silverfish has a lactase also.

In *A. collaris* restriction to a particular class of food does not apparently prevent secretion of enzymes specific for other foods, for even on a mainly protein diet the carbohydrases are active. Similarly, Day & Powning (1949) showed for the cockroach, *Blattella germanica*, that "all the enzymes are secreted irrespective of the nature of the food".

The detection of carbohydrases in anterior stomodaeum/salivary tissue and in the proctodaeum, and the non-detection of most of the other enzymes in these regions, might well be due to a higher degree of sensitivity of the tests for carbohydrases.

Using fibrin as substrate, Wall & Swift (1954) did not detect pepsin in *Thermobia domestica*. In *A. collaris* also there was only a slight digestion of fibrin at low pH, and that only by the crop. However, digestion of casein actually seems to be possible in crop and mesenteron, under pH conditions which suggest the activity of a pepsin-like proteinase. The acidity obtaining in the gut of *A. collaris* (Modder, 1962) points to the possession of a set of enzymes capable of functioning in acidic conditions, and therefore it seems logical to expect the presence of an acid proteinase, as has been reported already in other insects (Pavlovsky & Zarin, 1922; Brown, 1928; Uvarov, 1928; Mackerras & Freney, 1933; Goodchild, 1952; Day & Waterhouse, 1953; Greenberg & Paretsky, 1955; Gilmour, 1961).

Digestion of fibrin under alkaline conditions is possible even after an initial subjection to low pH. The same possibility was shown by Wigglesworth (1928) in cockroaches, and by Roy (1937).

While most of the protein digestion occurs in crop and mesenteron, "erepsin" activity in the proctodaeum as well might ensure a completion of breakdown of the products of proteinase action which might pass into the proctodaeum.

The distribution of the enzymes shows that the crop and the mesenteron are the main sites of digestion. As was previously suggested also (Modder, 1962), these two regions probably function together in digestion, because all the enzymes are generally found in both crop and mesenteron.

## SUMMARY

(1) Qualitative tests for detecting digestive enzymes in separate regions of the alimentary system of *Acrotelsa collaris*, made *in vitro*, are described.

(2) All the tests, including those for $\beta$-galactosidase (lactase) and a pepsin-like proteinase which were not found in *Thermobia domestica*, gave positive results.

(3) *A. collaris* is similar to cockroaches in being omnivorous; in possessing a lactase; in that, apparently, all its enzymes are secreted whatever the diet; and in that its trypsin-like proteinase can function after being subjected to low pH.

(4) The presence of an acid proteinase is to be expected in view of the acidic conditions in the gut.

(5) "Erepsin", found in the proctodaeum, might complete protein digestion there.

(6) The enzyme distribution shows that most of the digestion occurs in crop and mesenteron, and that these two regions function together.

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## REFERENCES

BALDWIN, E. AND BELL, D. J. 1955—*Cole's Practical Physiological Chemistry*. 10th Edn. Cambridge.

BROWN, F. M. 1928—Enzymes and bacteria in the honey bee. *American Mus. Novitiates*, No. 304.

DAY, M. F. AND POWNING, R. F. 1949—A study of the processes of digestion in certain insects. *Australian J. Sci. Res.* B, 2: 175—215.

DAY, M. F. AND WATERHOUSE, D. F. 1953—The mechanism of digestion. In *Insect Physiology* (edited by K. D. Roeder). New York. 311—30.

GILMOUR, D. 1961—*The Biochemistry of Insects*. New York & London.

GOODCHILD, A. J. P. 1952—A study of th digestive system of the West African cacao capsid bugs (Hemiptera, Miridae). *Proc. zool. Soc. Lond.* **122**: 543—72.

GREENBERG, B. AND PARETSKY, D. 1955—Proteolytic enzymes in the house fly, *Musca domestica* (L.). *Ann. ent. Soc. America* **48**: 46—50.

LASKER, R. AND GIESE, A. C. 1956—Cellulose digestion by the silverfish *Ctenolepisma lineata*. *J. exp. Biol.* **33** : 542—53.

MACKERRAS, M. J. AND FRENEY, M. R. 1933—Observations on the nutrition of maggots of Australian blow-flies. *J. exp. Biol.* **10** : 237—46.

MODDER, W. W. D. 1962—Hydrogen ion concentration in the alimentary canal of *Acrotelsa collaris* (Thysanura: Lepismatidae). *Ceylon J. Sci.* (*Bio. Sci.*) **4** (2) : 77—87.

PAVLOVSKY, E. N. AND ZARIN, E. J. 1922—On the structure of the alimentary canal and its ferments in the bee (*Apis mellifera* L.). *Quart. J. micr. Sci.* **66** : 509—56.

ROY, D. N. 1937—The physiology of digestion in larvae of *Gastrophilus equi*. *Parasitology*, **29** : 150—62.

SPENCER, G. J. 1930—The fire brat, *Thermobia domestica* Packard, (Lepismidae) in Canada. *Canadian Entomologist* 62 : 1—2.

SWINGLE, H. S. 1925—Digestive enzymes of an insect. *Ohio J. Sci.* **25** : 209—18.

TRAGER, W. 1932—A cellulase from the symbiotic intestinal flagellates of termites and of the roach, *Cryptocercus punctulatus*. *Biochem. J.* **26** : 1762—71.

UVAROV, B. P. 1928—Insect nutrition and metabolism. *Trans. ent. Soc. London* **76** : 255—343.

VARLEY, H. 1962—*Practical Clinical Biochemistry*. 3rd Edn. London & New York.

WALL, J. W., Jr. AND SWIFT, A. H. P. 1954—The digestive enzymes of the firebrat. *J. econ. Ent.* **47**: 187—8.

WIGGLESWORTH, V. B. 1927—Digestion in the cockroach. II. The digestion of carbohydrates. *Biochem. J.* **21** : 797—811.

WIGGLESWORTH, V. B. 1928—Digestion in the cockroach. III. The digestion of proteins and fats. *Biochem. J.* **22** : 150—61.

WIGGLESWORTH, V. B. 1953—*The Principles of Insect Physiology*. 5th Edn. London.

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