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A STUDY ON THE PHYSIOLOGY OF DIGESTION IN *MARINOGAMMARUS MARINUS* LEECH

By V. P. AGRAWAL

Zoology Department, D.A.V. College, Muzaffarnagar. (U.P.)

INTRODUCTION

THE amphipod, *Marinogammarus marinus* Leech, is a true littoral form, being abundant at high water level, normally found amongst the dark fuci which grow with much fertility between the tide marks. A full grown animal is about 16 mm. long.

Marinogammarus marinus is a macrophagus and raptatory feeder; it feeds entirely on plant material by picking up large particles of food with the help of antennae, maxillipeds and gnathopods, while mandibles help in crushing the food particles.

Sars (1895) has given the distribution of the species and has also briefly described its mouth parts. However, no work has so far been done on the alimentary canal of this amphipod. It may also be recalled here that none of the amphipod has been studied with respect to its physiology of digestion.

In this present paper the alimentary canal of *M. marinus* has been briefly described. The hydrogen-ion-concentration in the different parts of the gut under different conditions has also been studied; the qualitative estimation of the different digestive enzymes in the different parts of the alimentary canal and associated glands of the animal has also been carried out.

MATERIAL AND METHODS

Living animals were collected in large numbers from the Whitstable sea coast, Great Britain, where they occur in abundance (Newell, 1954).

In order to study the structure of the alimentary canal of *M. marinus*, the alimentary canal was reconstructed and set into the predrawn outline diagram of the animal with the help of the serial transverse sections.

To observe the passage of the food, a few starved animals were kept in a dish with a sludge of carmine particles for half an hour; these were then removed to another dish containing distilled water. The animals were dissected after a series of interval of 5 minutes and the location of carmine in each case was determined.

The pH in the alimentary canal of the animal was determined in the freshly collected specimens, in starved animals and in the animals which were first starved for 40 hours and then fed on selected diet such as *Ulva* frond. As the quantity of secretion available from the different parts of the gut is very small, the capillary

indicator method and the pH meter method were abandoned in favour of indicator paper method. The different parts of the alimentary canal of *M. marinus* were slightly teased and brought in contact with the indicator papers, the colours of which were compared with a standard series of colours and the pH was calculated.

For the preparation of extracts of foregut, midgut, hindgut and the ventral hepato-pancreatic caeca, as many as 200 specimens were dissected and the different parts of the gut were carefully separated and thoroughly washed to remove any food contents ; these were, then ground up separately with a little thymol and a few drops of glycerol until a fine uniform emulsion was formed. Emulsion from each part was diluted to about 10ml with 50% glycerol and centrifuged at 3000 r.p.m. The supernatants were separated and kept in tubes and preserved with toluene. The extracts were kept at room temperature for about 48 hours before being tested for the different enzymes.

A few drops of each extract were incubated at room temperature with the addition of a few drops of the different substrates. The control experiments were also set up in each case. These incubated solutions were tested after the intervals of 24 hours, 48 hours and 4 days for any digestion of the substrates. For the determination of amylase, invertase, glycogenase, raffinase, inulinase, salicinase and amygdalinase, Fehling's and Benedict's tests were performed while for maltase and lactase, osazone and Barfoed's tests were performed.

To test for the presence of cellulase, a 1% fucoid solution, a 1% Laminarin solution and a 1% solution of sodium carboxy methyl cellulose were separately incubated with a concentrated solution of the caecal extract prepared in distilled water. In each case the hydrolysis of cellulose was tested by Benedict's and Fehling's solutions.

The presence of lipase was tested by conducting experiments on condensed milk. Two drops of bromo-thymol blue were added to 25 ml. of a 10% solution of milk ; 1% sodium hydroxide solution was added until the solution turned light blue. One ml. of blue milk solution and a few drops of the extracts from the different parts of the gut were incubated in the tubes filled with toluene and the change in the colour was noted after different intervals. These results were confirmed by performing experiments with olive oil. 10 drops of olive oil were dissolved in 4 ml. of absolute alcohol ; 4 ml. of hot water was added to it. The mixture was then allowed to cool and 10 drops of phenol red were added. A few drops of 0.01 N NaOH were added to make the emulsion faintly pink. 2 ml. of this mixture was incubated with 1 ml. of the different extracts at room temperature.

OBSERVATIONS

Alimentary Canal and Feeding in *M. marinus*

The alimentary canal of *Gammarus pulex*, a fresh water species has been described by Cussan (1904) ; the alimentary canal of this marine species differs but little with that of *G. pulex*.

The alimentary canal of *M. marinus* consists of the foregut, midgut with hepatopancreatic caeca and the hindgut. The foregut and hindgut constitute the stomodaeum and proctodaeum respectively and are lined internally with cuticle while the midgut forms the mesentron. The foregut includes the oesophagus and the cardiac and pyloric parts of the stomach. The inner wall of the cardiac stomach is produced into paired dorso-lateral, latero-lateral and ventro-lateral

ridges which are beset with a large number of teeth-like spines and hooks and thus serve for the trituration of food. Posteriorly, the ridges of the cardiac stomach, before it passes into the pyloric stomach, become lamelliform and extend a long way into the lumen and incompletely divide it into a small dorsal portion, a large middle chamber and a narrow ventral chamber. The pyloric part of the stomach is provided with a large number of fine bristles and serves for the filtering of food particles.

The midgut at its junction with foregut gives out a single median dorsal caecum which runs forward above the stomach and a pair of ventral caeca which run backward and soon divide into two pairs, running on either side of the midgut almost upto the end of the midgut. Paired posterior dorsal caeca arise from the posterior part of the midgut and run forward on either side of the midgut. Small rectum opens dorsally by the narrow anus.

The feeding experiments revealed that *M. marinus* feed on vegetable diet ; a few of the starved animals when tempted with animal diet such as small pieces of mussels, rejected to feed on it. A few animals were also kept with soaked filter paper and they were found to feed on it. However, these animals died after about 5 hours and their guts were found to be full of long fibres of filter paper which they are not able to digest or absorb.

The experiments on the passage of the food showed that the oesophagus and most of the stomach was full of carmine ; the carmine was first noticed in the midgut after 40 minutes. It was only after two and a half hours that the carmine first passed out through the anus.

pH Determination in the different parts of the Alimentary Canal

The determination of pH in the different parts of the digestive tract is an important aspect of the physiological studies, for different enzymes act optimally under different hydrogen-ion-concentrations.

In the Table I below, the pH readings in the different parts of the gut of *M. marinus* are shown along with the averages.

TABLE I

pH in the different parts of the gut of *M. marinus*.

S. No.	Ventral caeca	Foregut	Midgut	Hindgut
1	6.50	6.70	6.60	6.80
2	6.40	6.70	6.50	6.90
3	6.40	6.80	6.75	6.90
4	6.50	6.70	6.80	6.85
5	6.40	6.80	6.85	6.80
6	—	6.75	6.90	6.80
7	—	6.65	6.80	6.80
8	—	6.70	6.75	—
Averages	6.45	6.70	6.85	6.75

The table shows that the average pH in the four parts of the gut, namely ventral caeca, foregut, midgut and hindgut of the normal feeding animal is more or less the same and is only slightly acidic. However, the ventral caeca, where the different digestive enzymes are secreted, is more acidic.

A few animals were starved for about 80 hours so as to clear off the alimentary canal, and the pH in the above mentioned four parts did not show appreciable difference from those shown in the above table. The pH in the animals which were first starved for 40 hours and then fed on *Ulva* frond also agreed with the above readings.

Similar experiments were conducted with another species of *Gammarus*, *G. pulex* which is a freshwater form; the averages of pH were found to be: ventral caeca=6.55; foregut=6.75; midgut=6.80 and hindgut=6.80. It may, therefore, be concluded that the pH in the gut and caeca of *G. pulex* agrees with that of *M. marinus*.

Qualitative Estimation of Enzymes

The experiments were designed with a view to locating the sites of secretions in the gut of *M. marinus*, and the results are set out in Table II.

TABLE II

The occurrence of different enzymes in the caecal extract of *M. marinus*.

Substrate	Duration of reaction and extent of digestion			Control experiments	
	24 hrs.	48 hrs.	4 days	24 hrs.	3 days
1% starch solution ..	++	++	++	−	−
Saturated soln. of glycogen ..	++	++	++	±	±
5% sucrose soln. ..	++	++	++	−	−
2% maltose soln. ..	+	+	++	−	−
2% lactose soln. ..	−	−	−	−	−
1% raffinose soln. ..	±	±	±	−	−
1% inulin soln. ..	±	±	±	−	−
1% salicin soln. ..	±	+	+	−	−
1% amygdalin soln. ..	+	+	+	−	−
1% fucoid soln.* ..	−	−	−	−	−
1% Laminarin soln.* ..	−	−	−	−	−
1% sodium carboxy* methyl cellulose ..	−	−	−	−	−
10% gelatine soln. ..	Completely digested after 8 hrs.			Remained solid	
Blue milk soln. ..	Colour changed to yellow			No change in colour	
Olive oil etc. ..	Colour changed			No change	

* Fucoid and Laminarin were obtained from the Seaweed Research Institute, while carboxy methyl cellulose was procured from I.C.I., England.

(The sign ++ means a vigorous reaction; + a definite positive reaction; ± traces of reaction and the sign − indicates no reaction.)

As a result of these experiments, it was found that none of the enzymes are present either in the foregut or in the midgut. However, most of the carbohydrates are present in the caecal extract of *M. marinus*; amylase, glycogenase, sucrase and maltase are particularly very active, while the lactase and cellulase are absent.

The presence of proteases was investigated by incubating a few drops of the different extracts with 10% gelatine solution and it was found that the gelatine was completely liquified with caecal extract while it remained solid with the foregut, midgut and hindgut extracts as well as with the control experiments. Proteases are, therefore, secreted by the caecal cells of the animal.

Experiments with blue milk and olive oil solution also showed that the lipase is quite active in the caecal extract of *M. marinus* while it is not present in any other part of the alimentary canal.

Discussion

It may be pointed out that the feeding appendages and the form of the gut are related to the character of the food taken. Under the character of the food, it has to be considered, whether it is predominantly animal or vegetable and also the size of the food. It has been found that *M. marinus* feeds entirely on plant material and it is macrophagous, feeding on large masses of food which are primarily masticated by the mandibles; further trituration of the food takes place in the cardiac stomach which is provided with a large number of chitinous hooks and spines. The pyloric stomach serves as a filter apparatus and also mixes food with the digestive enzymes. The food then passes into the midgut where it is digested and absorbed as in *Orchestia gammarella* (Agrawal, 1961a). Ide (1892) while discussing the functions of the stomach in different crustacea also points to the masticatory function of the cardiac stomach and filtering mechanism of the pyloric stomach.

It has been found that the pH in the different parts of the gut of *M. marinus* is very similar to each other and is nearer neutrality, being only slightly acidic. Similar investigations, by the author, on *Orchestia gammarella* (unpublished work) and *Corophium volutator* (Agrawal, 1962) revealed that the medium in the ventral caeca is distinctly acidic while in the gut proper it is only very weak acid.

The ventral hepato-pancreatic caeca alone of *M. marinus* like that of *O. gammarella* (Agrawal, 1961b) secrete the digestive enzymes and also serve for the absorption and storage of reserve food material in the form of oil globules. Nicholls (1931) in his work on *Ligia* also observed that the ventral caeca subserve the functions of digestion and absorption of food. Thus the hepatopancreas, as the name signifies, share the functions of liver and pancreas of higher chordates.

Studies on the type of food, i.e., animal or vegetable with relation to the enzyme equipment and physiology of digestion in *C. volutator*, *O. gammarella* and *M. marinus* shows that most of the carbohydrates are digested by the caecal extracts of *O. gammarella* and *M. marinus* as could be expected in animals which feed on a variety of animal or plant diet, while among the carbohydrates, only starch, glycogen, sucrose and maltose are digested by *C. volutator* (Agrawal, 1962) which

is a selective feeder and according to Crawford (1937) consumes only a small proportion of suspended particles.

It has also been experimentally investigated that *O. gammarella* (Agrawal, 1961b) which eat all dead organic matter, both animal and vegetable and even paper and cloth, are able to feed and digest the cellulose, while starved animals of *M. marinus*, although greedily feed on soaked filter paper, soon die. On dissection of these dead animals, it was found that their gut was full of large fibres of cellulose which they were not able to digest.

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Summary

Marinogammarus marinus is a true littoral form and is a macrophagous and raptatory feeder.

The cardiac stomach of the animal supplements for the mastication of food while the pyloric stomach acts as a filter apparatus.

The study of pH in the different parts of the gut of *M. marinus* shows that it is very similar with each other and is only slightly acidic. However, the medium in the ventral caeca is more acidic.

Studies on the qualitative estimation of the digestive enzymes in the parts of the gut show that the ventral caeca alone secrete the different enzymes. Most of the carbohydrates are digested by the caecal extract; the cellulose, on the other hand, is not digested. Active proteases and lipase are also secreted by the caecal cells of *M. marinus*.

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