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ABSORPTION ALONG THE ALIMENTARY TRACT OF BARNACLES (CIRRIPEDIA: THORACICA)

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(Plate I)

Absorption occurs in all regions of the midgut of sessile and stalked barnacles, but it is most significant in the anterior midgut and caeca. ^{14}C-labelled bicarbonate, incorporated into the diatom *Chaetoceros calcitrans* (food source), was taken up by the midgut epithelium of *Balanus balanoides* (L.) before being transported across the *stratum perintestinale* layer to the prosomal parenchyma tissue where storage occurred. Labelled material was then redistributed to other body regions such as the pancreatic glands. *B. balanoides* feeding on diatoms produced faecal pellets after approximately 2 h and thereafter at 20 min intervals. Ingested horseradish peroxidase was absorbed in all regions of the midgut of the stalked barnacles, *Lepas anatifera* and *Pollicipes mitella*, absorption being greatest anteriorly.

INTRODUCTION

Rainbow & Walker (1977) have described the histology, histochemistry and ultrastructure of the barnacle alimentary tract and this study continues the overall investigation into barnacle digestive physiology. Possible sites of alimentary absorption in the barnacle tract have been proposed from histological (see Törnävä, 1948) and histochemical and ultrastructural evidence (Rainbow & Walker, 1977) but no direct studies on sites of absorption have previously been made. Similarly, although it has long been known that barnacles produce discrete faecal pellets (Darwin, 1851), the time interval between ingestion and defaecation has not been determined.

MATERIALS AND METHODS

Autoradiography

Balanus balanoides (L.), attached to small stones, were collected from MTL in the Menai Strait and placed in filtered sea water for 3 days. A culture of the diatom *Chaetoceros calcitrans* (Paulsen) Takano was set up in 200 ml Erd Schreiber medium at room temperature, and after 2 days, 2 ml of carbonate/bicarbonate buffer (pH 9·9) containing 105 μCi of ^{14}C-labelled $NaHCO_3$ (The Radiochemical Centre, Amersham) were added. The culture was allowed to grow for a further 2 days so that the alga passed through the exponential growth phase, ensuring maximum incorporation of the radioactive label. The labelled culture was then divided into two crystallizing dishes each containing some of the barnacles, whilst unlabelled *C. calcitrans* (control) was added to a third crystallizing dish with the remaining barnacles. The dishes were maintained at room temperature and the contents were well aerated. Barnacles were detached from the stones in the two experimental dishes after ½, 2, 3½, 5 and 24 h and their bodies fixed in Clarke's fixative (Carnoy's fluid without chloroform). Bodies of barnacles from the control dish were fixed in Clarke's after 2 h.

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After fixation and dehydration, all specimens were embedded in ester wax and sectioned at 7 μm; the wax sections were floated onto subbed slides (see Rogers, 1969). After dewaxing, the sections were brought to distilled water before being taken into the darkroom (Wratten No. 2 filter). Kodak AR 10 stripping film was wrapped around the slides as described by Rogers (1969) and, when dry, the filmed slides were placed in a light-tight box, containing silica gel as desiccant, and placed in a refrigerator at 4 °C. After 12 days exposure, the filmed slides were developed in Kodak D 19 developer and fixed (Rogers, 1969). Some slides were processed without staining, others were post-stained using the following variation of the technique of Doniach & Pelc (1950): (1) well-washed in running tap water; (2) stained for 5 min in Mayer's haemalum; (3) dipped in 1 % HCl; (4) placed in running tap water, 15 min; (5) dipped in 2 % aqueous polyvinyl alcohol; (6) air-dried; (7) dehydrated 2 × absolute alcohol; (8) cleared in xylene; mounted in D.P.X. Processed autoradiographs were viewed with dark field illumination.

Zinc phosphate granules in cells beneath the midgut epithelium (see Walker *et al.* 1975*a*, *b*) were highlighted in unstained sections but were usually dissolved away during any staining.

Histochemistry

Specimens of *B. balanoides* were collected from the Menai Strait during May, and processed as described by Rainbow & Walker (1977) before staining with Periodic-Acid Schiff's (PAS) and Best's carmine (using diastase digestion as control).

Faecal pellet production

Small stones collected from the shore of the Menai Strait were scraped clear of all settled organisms except for a single specimen of *B. balanoides*. Such barnacles were maintained without food for 3 days and then placed in fresh filtered sea water in crystallizing dishes. A culture of the diatom *Skeletonema costatum* (Grev.) Cleve was filtered (plankton netting; 45 μm pore size) to remove any large clumps of algae, and added to the dishes which were well aerated at 15 °C. The dishes were inspected at 15 min intervals over a 6 h period and the faecal pellets produced were removed and measured.

Horseradish peroxidase

Pollicipes mitella (L.), collected from Big Wave Bay, Hong Kong, and *Lepas anatifera* (L.), washed up on the west coast of Anglesey, were maintained in the laboratory at 20 °C.

Pieces of adductor muscle of the horse mussel, *Modiolus modiolus* (L.), were soaked in aqueous horseradish peroxidase solution (25 mg/ml) for 24 h at 4 °C. These muscle pieces were then offered to the stalked barnacles which were each fed over a 15 min period (control barnacles were fed untreated muscle tissue). After a further period of 3 h, the bodies of *L. anatifera* and *P. mitella* were dissected out and fixed for 30 min in 4 % formalin in sea water at 4 °C, rinsed in distilled water and quenched in liquid nitrogen. Sections were cut at 10 μm in a cryostat and incubated for 30 min in 10 ml of 0·5 M Tris-HCl buffer (pH 7·6), containing 10 mg 3-3′-diaminobenzidine tetrahydrochloride and 0·02 % H_2O_2. Sections were then washed briefly in distilled water and mounted in glycerine jelly.

RESULTS

Autoradiography: Balanus balanoides

½ *hour*. Radioactive material was present in the lumen of the midgut, activity being strongest in the lumen of the anterior midgut. The epithelial cells of the anterior midgut, midgut caeca and posterior midgut also showed activity (Pl. 1A), most activity being present in the cells of the anterior midgut and caeca. Weak activity was sometimes present in those tissues immediately beneath the midgut epithelium (the *stratum perintestinale* layer, see Törnävä, 1948; Rainbow & Walker, 1977).

2 *hours*. Strong activity was present in the midgut lumen and again in the epithelial

Table 1. Balanus balanoides: *faecal pellet production of three individuals (I, II and III) fed on* Skeletonema costatum *at* 15 °C.

	Period of faecal pellet production				
Barnacle	Period of feeding until 1st faecal pellet produced (min)	Number of faecal pellets produced	Time after extrusion of initial faecal pellet (min)	Average time/faecal pellet (min)	Average length faecal pellet (mm)
I	135	15	225	15	1·219
	105	14	255	18·2	
	120	15	240	16	
II	120	13	240	18·5	1·242
	165	11	195	17·7	
III	120	5	240	48	1·378
	105	3	255	85	
Mean	124·3			21·7	1·240

cells of all regions of the midgut. The underlying prosomal parenchyma tissue contained only weak activity.

$3\frac{1}{2}$ *hours*. Whilst the midgut lumen still contained strong activity, the level of activity in the parenchyma cells had increased. However, the levels of activity in the midgut epithelial cells and the *stratum perintestinale* cells (Pl. I C, D) remained similar to those seen after 2 h. Other organs and tissues, such as the pancreatic glands and muscles, contained no activity.

5 hours. Activity in the midgut lumen was still strong, as was that in the prosomal parenchyma tissue (Pl. I E, F). The epithelial cells of all midgut regions contained activity at a level similar to that in $3\frac{1}{2}$ h animals and still only slight activity was present in the *stratum perintestinale* cells (Pl. I B). Muscles still showed no activity but the pancreatic glands did now contain weak activity.

24 *hours*. The contents of the midgut lumen continued to show strong activity and the midgut epithelial cells had increased activity in comparison with the previous levels which had remained relatively constant. The activity in the prosomal parenchyma tissue had not increased and that of the *stratum perintestinale* cells was still relatively weak. Most other organs such as muscles, maxillary (excretory) glands and foregut, in addition to the pancreatic glands, also contained activity. Activity was particularly evident in the nuclei and apical cytoplasm of the pancreatic gland cells (Pl. I G, H).

Histochemical studies: B. balanoides

Plate I J, K shows the distribution of glycogen around the midgut of *B. balanoides* collected in May. The prosomal parenchyma tissue contains large glycogen deposits which, although present to a lesser degree in the midgut epithelial cells, are notably absent from the *stratum perintestinale* cells (see Rainbow & Walker, 1977).

Faecal pellet production and the time of passage of food material through the alimentary tract

Specimens of *B. balanoides* produced their first faecal pellets approximately 2 h after the commencement of feeding (see Table 1). Following this initial period, the faecal pellets were extruded at approximately 20 min intervals. The average length of a faecal pellet is about one-third of the average length of the midgut of adult *B. balanoides* (4113 μm; Rainbow, unpublished). Thus, at room temperature, in barnacles feeding on diatoms such as *Skeletonema*, the contents of the midgut are probably renewed each hour.

Absorption of horseradish peroxidase in stalked barnacles

Three hours after feeding, specimens of *Lepas anatifera* contained horseradish peroxidase in the epithelial cells of the anterior midgut, the midgut caeca and the posterior midgut. Horseradish peroxidase was found throughout the cytoplasm of the anterior midgut and caecal epithelial cells, but was restricted to the apical cytoplasm in the posterior midgut cells. *Pollicipes mitella* lacks midgut caeca (see Darwin, 1851), but the distribution of horseradish peroxidase is essentially similar to that in *L. anatifera*. The absorbed enzyme is found throughout the cytoplasm of the anterior midgut cells, and is also found to a lesser extent, restricted apically, in the posterior midgut cells.

DISCUSSION

Balanus balanoides (*sessile barnacle*)

It is apparent from the autoradiographic study that all regions of the midgut absorb digested food material to a significant degree, as was suggested by Rainbow & Walker (1977) from histochemical evidence. The cells of the anterior midgut and caeca may play the major role in absorption, simply because they form the larger surface area.

The temporal distribution of absorbed carbon-14 gives a good indication of the route of translocation of absorbed material. The midgut epithelial cells contained labelled material within half an hour of food ingestion and, after 2 h, the labelled material had reached the prosomal parenchyma tissue. This tissue contained high levels of absorbed material after 3–5 h, whilst the midgut epithelial cells maintained a lower (apparently constant) level of activity. Interestingly, the *stratum perintestinale* cells consistently contained the lowest activity level when compared with surrounding tissues. The morphological distribution of activity after 3–5 h is very similar to the histochemical distribution of glycogen in *B. balanoides* collected in the summer. High levels of glycogen are stored in the parenchyma cells, a lower level is present in the midgut epithelium and the *stratum perintestinale* cells are devoid of glycogen.

It is possible therefore that much of the ^{14}C-labelled bicarbonate, incorporated in the diatom *Chaetoceros calcitrans*, becomes labelled carbohydrate. Whilst such carbohydrates, after digestion and translocation, are eventually stored as glycogen in the prosoma, it is probable that primary digestion products are simple sugars which are leached out from both the midgut epithelium and *stratum perintestinale* layer during the fixation and preparation of sections, leaving these layers with their observed low levels of activity.

Approximately 5 h after the start of feeding, some absorbed digestive products have

been transported to the pancreatic gland cells which are secretory (Rainbow & Walker, 1977). After 24 h, absorbed materials are distributed throughout the body tissues, being present for example in muscles. The distribution of activity within the pancreatic gland cells after 24 h indicates incorporation of newly absorbed materials into the nucleus and into the apical cytoplasm, thence into the gland lumen. The activity in the midgut epithelial cells after 24 h has increased relative to the activity present after the shorter feeding periods, although this latter activity had been constant for some hours. It is possible that absorbed materials may now have been transported back to the midgut epithelium to take part in the metabolism of the cells, the rate of absorption of active material from the midgut lumen remaining constant.

Whilst studies on faecal pellet production have shown that the barnacles might renew the contents of the midgut at short intervals (1 h), the autoradiographic studies have confirmed that considerable absorption occurs in the alimentary tract in spite of the rapid throughput.

Stalked barnacles

Whereas sessile barnacles, such as species of *Balanus*, withdraw beneath the opercular valves when approached by large objects, including potential food material (Crisp & Southward, 1961), stalked barnacles (e.g. *Lepas*, *Pollicipes*) may grasp such objects by one or more cirri and ingest them (Barnes & Reese, 1959; Howard & Scott, 1959). It was therefore possible to feed the stalked barnacles with pieces of tissue soaked in horseradish peroxidase.

In *L. anatifera* and *P. mitella*, horseradish peroxidase is absorbed by both anterior and posterior midgut cells (also by the caecal cells of *L. anatifera*). More of the enzyme was found anteriorly in the midgut in each case and so the anterior midgut (plus caeca if present), is probably again the major area of absorption.

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EXPLANATION OF PLATE I

Abbreviations: a.m.g., anterior midgut; e., epithelium; g., granules of zinc phosphate; l., lumen; n., nuclei of pancreatic gland cells; p.g., pancreatic gland; p.m.g., posterior midgut; p.p., prosomal parenchyma; s.p., *stratum perintestinale*.

(A) *Balanus balanoides:* a section of the anterior midgut after ½ h feeding on labelled diatoms (dark field illumination). Scale is 60 μm.

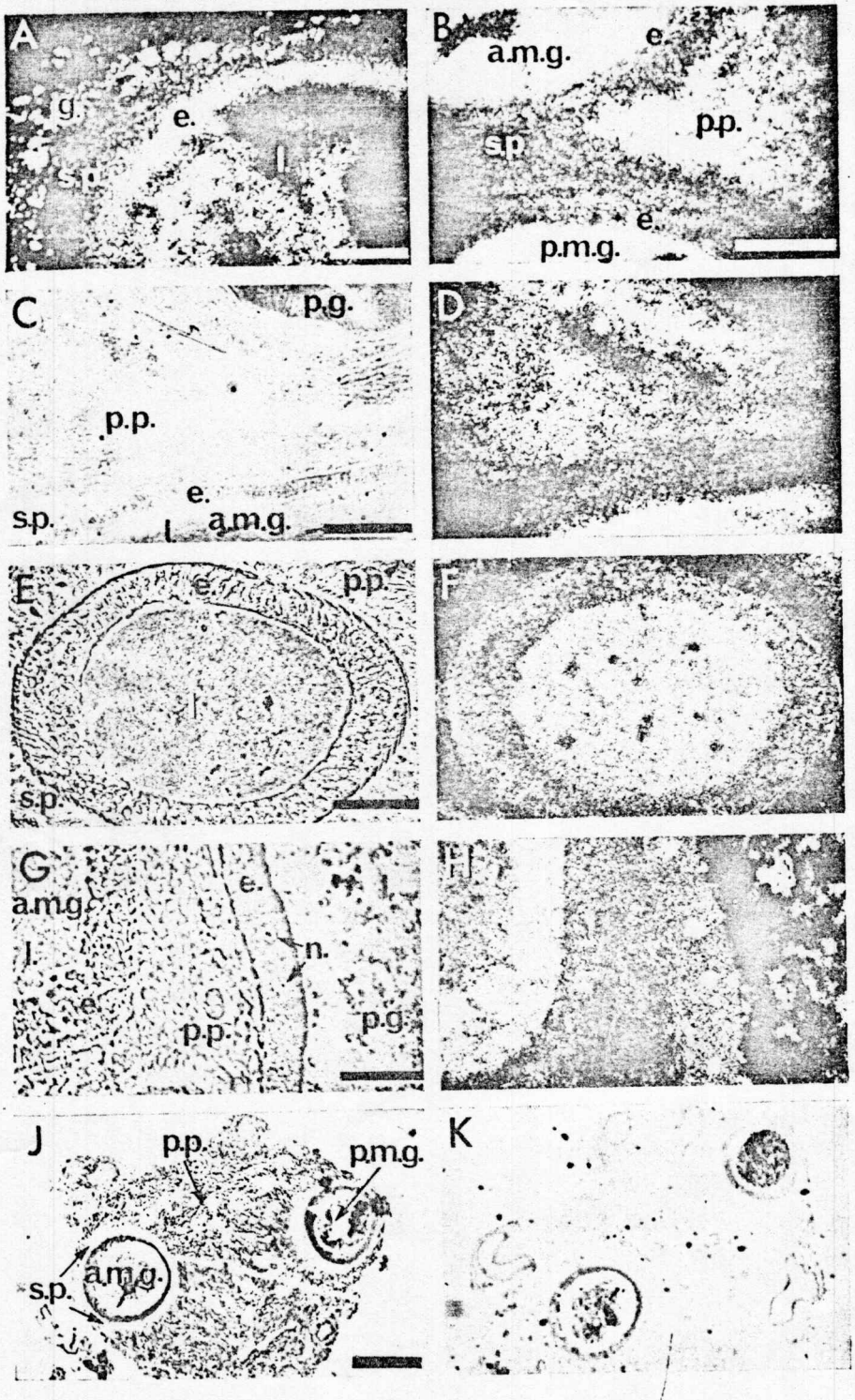
(B) *Balanus balanoides*: a section through the prosoma after 5 h feeding on labelled diatoms (dark field illumination). Scale is 75 μm.

(C, D) *Balanus balanoides*: (C) a section through the anterior midgut (a.m.g.) and pancreatic gland (p.g.) after 3½ h feeding on labelled diatoms (stained with Mayer's haemalum); (D) same section with dark field illumination showing the distribution of label. Scale is 75 μm.

(E, F) *Balanus balanoides*: (E) a section through the posterior midgut after 5 h feeding on labelled diatoms (phase contrast); (F) same section with dark field illumination showing the distribution of label. Scale is 75 μm.

(G, H) *Balanus balanoides*: (G) a section through the anterior midgut (a.m.g.) and pancreatic gland (p.g.) after 24 h feeding on labelled diatoms (stained with Mayer's haemalum); (H) same section with dark field illumination. Scale is 75 μm.

(J. K) *Balanus balanoides*: (J) a section through the prosoma stained for glycogen (Best's carmine); (K) control section similarly stained but pretreated with diastase. Glycogen is present throughout the prosomal parenchyma (p.p.) but is conspicuously absent from the *stratum perintestinale* (s.p.) (animal fixed May 1973). Scale is 240 μm.



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