Histological Changes in the Gut of *Mitopus morio* (Phalangiida) during Protein Digestion

By JOHN PHILLIPSON

(*From the Department of Zoology, Durham Colleges, University of Durham*)

Summary

1. The description of the gross morphology of the gut of *Mitopus morio* agrees with Kästner's (1933) description for *Opilio parietinus* De Geer except that 4 pairs of lateral openings lead from the mid-gut into the diverticula and not 3 as previously recorded.

2. The two main types of diverticular cell in *M. morio* are digestive and secretory. There is thus agreement with the structures present in spiders, scorpions, and other harvest-spiders.

3. The mid- and hind-gut epithelia both possess a single cell-type. That of the mid-gut is absorptive and digestive, whereas that of the hind-gut secretes a membrane round the faeces to form a pellet.

4. During protein metabolism some extracellular digestion occurs in the lumina of the diverticula and mid-gut. The necessary enzyme or enzymes are produced by the diverticular secretory cells and are replenished within 24 h.

5. Extracellular digestion is followed by partial or complete intracellular digestion in the diverticular digestive cells, which then cut off either digestive cell apices, containing partially digested protein globules, or digestive cell faeces which contain excretory material.

6. Further digestion of the protein globules of the digestive cell apices occurs in the mid-gut cells, which then produce mid-gut cell faeces that are voided with the digestive cell faeces and indigestible food remnants.

Introduction

THERE is a close similarity in the gut structure of all Arachnida (Grassé, 1949) and a similar agreement in function of the cell components of the gut might well be expected. To some extent this has been established. Thus Kastner (1935) and Frank (1937) showed the two main types of cell in the mid-gut diverticula of harvest-spiders to be digestive and secretory, which agrees with the descriptions by Millot (1926) for spiders and Pavlovsky and Zarin (1926) for scorpions. However, Gilbert (1952) working on Chelonethi (pseudo-scorpions) showed that the diverticular cells were of two types, digestive or excretory. The problem therefore arises whether there has been a misinterpretation of cell function in the other groups. In view of these divergences, the histology of the gut system in harvest-spiders has been re-investigated by Gilbert's (1952) techniques. This has entailed a description of the general morphology of the gut in *Mitopus morio* and a study of the histological changes occurring during protein digestion.

[Quarterly Journal of Microscopical Science, Vol. 102, part 2, pp. 217–26, June 1961.]

Materials and Methods

The adult *M. morio* (F) used in this work were collected by hand in the vicinity of Durham City and maintained in the laboratory in accordance with Phillipson's (1960) directions.

The general morphology of the gut was determined by dissecting specimens (fixed in 95% alcohol) under a stereoscopic microscope with fine needles. Details were obtained from serial sections of animals fixed in Susa, embedded in paraffin wax (melting-point 54° C), cut at 8 μ, and stained in haematoxylin and eosin.

Changes in the gut epithelia during protein digestion were determined on animals kept individually, each fed on one *Scopeuma stercoraria* female and then starved for 3 days. They were then given a further feed of the same food and fixed at intervals of $\frac{1}{2}$ h, 2 h, 6 h, 12 h, 18 h, 1 day, 2 days, 4 days, and 6 days after feeding. At least two individuals were used for each observation and Gilbert's (1952) techniques were employed.

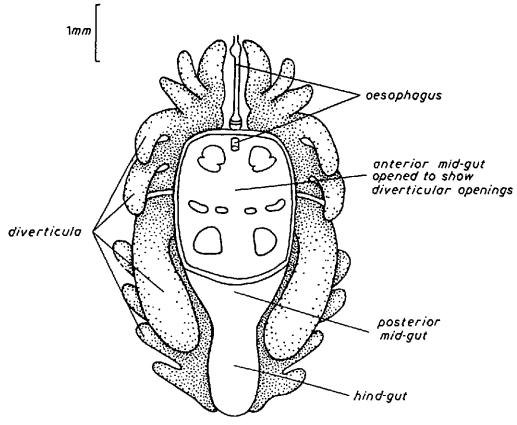

Fig. 1. *Mitopus morio*. General morphology of the gut (ventral view).

Morphology of the Gut

Kästner (1934) showed that the morphology of the gut of harvest-spiders differed between families. The Phalangiidae, to which *M. morio* belongs, has the most complex system of mid-gut diverticula. Fig. 1 shows that the narrow, chitin-lined oesophagus expands into a capacious mid-gut which is divided into anterior and posterior regions. It is clear from both sections and dissections of *M. morio* that 4 pairs of openings lead into the diverticula from the anterior mid-gut and not 3 pairs as stated by Kästner (1933) for *Opilio parietinus* De Geer and *Phalangium opilio* L. The posterior region is not perforated

by diverticular openings, and the hind-gut, divided from the mid-gut by a constriction, is short.

Histology of the Gut

The anterior mid-gut is lined with a uniform columnar epithelium composed of one cell-type. The posterior mid-gut, although lined with cells of the same size and structure as those of the anterior mid-gut, has its epithelium produced into 'papillae' which project into the lumen. In the diverticula (fig. 2, A) two

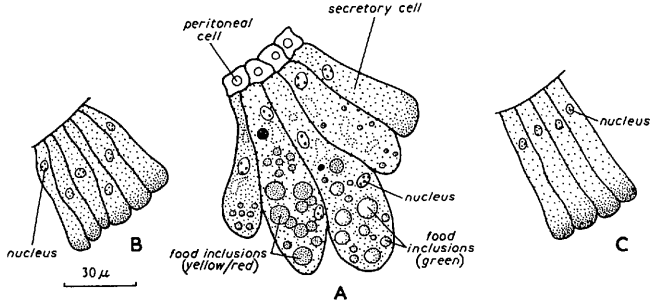

Fig. 2. A, wall of diverticular mid-gut. B, mid-gut cells. C, hind-gut cells. All 30 min after feeding. Susa: haemalum / acid fuchsin / metanil yellow / light green.

main types of cell can be distinguished, both differing from those in the mid-gut epithelium. One type, the digestive cell, is binucleate and occurs in the ratio of approximately two to every secretory cell. A uniform columnar epithelium of yet another cell type lines the hind-gut.

Table 1 summarizes the results obtained during protein digestion by staining with the modified quadruple method of Gilbert (1952). The reagents are haemalum which stains nuclei blue, light green which stains the ground cytoplasm green, and metanil yellow and acid fuchsin, both of which stain cell inclusions. The inclusions, which are believed to be protein, show a progressive change in their staining reaction from green to yellow-red, to red, and finally red-brown. The changing colour reaction is generally accepted to indicate different stages in protein metabolism.

At $\frac{1}{2}$ h and 2 h after feeding, although columnar diverticular digestive cells occurred (fig. 3, A), they were of the type described as 'young cells' by Frank (1937) and not equivalent to the later 'columnar' cells.

Sections stained with Heidenhain's iron haematoxylin, apart from coloration, gave identical results with those described above, except that in the Heidenhain series rod-shaped structures about 12 μ long and small globules some 0·75 μ in diameter occurred, at all feeding stages, in the cells surrounding that part of the oesophagus which projected into the mid-gut lumen. The

TABLE 1. *Summary of results obtained from sections stained by the quadruple method*

Cell type	*Size and shape of cell*	*Cell appearance at intervals (in hours) after feeding*								
		½	2	6	12	18	24	48	96	144
Diverticular secretory	60 μ high 25 μ wide Ovoid when fully developed	Fig. 2, A No inclusions	Fig. 3, A No inclusions	Fig. 4, A Small yellow/red globules	Numerous small yellow/red globules	Fig. 6, A Numerous large yellow/red globules	Filled with yellow/red globules	Fig. 7, A Filled with yellow/red globules	Packed with yellow/red globules	Fig. 8, A Packed with yellow/red globules
Diverticular digestive (d.d.)	90–100 μ high 30–35 μ wide Clavate	Fig. 2, A Mainly green and yellow/red globules	Fig. 3, A Mainly yellow/red and red globules	Fig. 4, A Yellow/red, red or red/brown globules in apical vacuole	Yellow/red, red or red/brown globules in apical vacuole	Fig. 6, A Red or red/brown globules in apical vacuole	Red or red/brown globules and granules in apical vacuole	Fig. 7, A Red/brown globules and granules in apical vacuole	Red/brown granules in apical vacuole	Fig. 8, A Red/brown granules in apical vacuole
	60–75 μ high 12–15 μ wide Columnar	See text	See text	Fig. 4, A No inclusions d.d. cell apices present	No inclusions d.d. cell apices present	Fig. 6, A No inclusions d.d. cell apices present	No inclusions Few d.d. cell apices and few d.d. cell faeces present	Fig. 7, A No inclusions Few d.d. cell apices but many d.d. cell faeces present	No inclusions Many d.d. cell faeces present	Fig. 8, A No inclusions Many d.d. cell faeces present
Mid-gut (m.g.)	45–60 μ high 6–12 μ wide Columnar	Fig. 2, B No inclusions	No inclusions	Fig. 4, B A few red globules Apical vacuoles	Fig. 5 Numerous red globules	Fig. 6, B Red globules aggregated in apical vacuoles	Red and red/brown globules in vacuoles	Fig. 7, B Red/brown particles in apical vacuoles Cell faeces present	No inclusions m.g. cell faeces present	Fig. 8, B No inclusions Cell faeces present
Hind-gut (h.g.)	60–75 μ high 15 μ wide Columnar	Fig. 2, C h.g. cell apices absent	Fig. 3, B h.g. cell apices present	h.g. cell apices present	h.g. cell apices present	h.g. cell apices present	h.g. cell apices present	h.g. cell apices present	h.g. cell apices present	h.g. cell apices present

Explanation of the terms used in table 1:

Diverticular digestive cells. d.d. cell apices = portion separated from the digestive cell and containing variously coloured globules (fig. 4, A); d.d. cell faeces = spheres separated from the digestive cells and containing red/brown stained granules (fig. 7, A).

Mid-gut cells. m.g. cell faeces = small spheres separated from the mid-gut cells and containing red/brown stained particles (fig. 7, B).

Hind-gut cells. h.g. cell apices = small spheres separated from the hind-gut cells and stained pink/brown (fig. 3, B).

rods and globules were also present in many of the mid-gut cells of animals which had been starved for 6 days (fig. 9, p. 226).

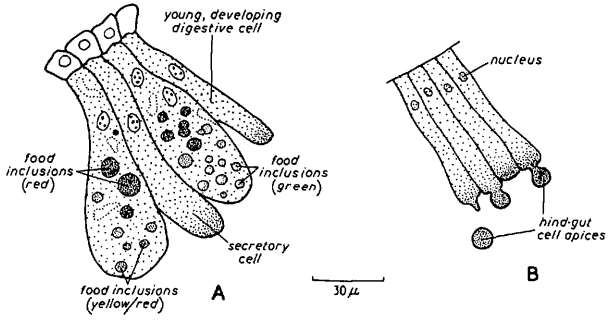

FIG. 3. A, one secretory and 3 diverticular digestive cells. B, hind-gut cells. Both 2 h after feeding. Susa: haemalum / acid fuchsin / metanil yellow / light green.

GUT CONTENTS

Unlike spiders and pseudoscorpions, which allow only liquid food to enter the gut, the harvest-spiders take in small solid particles as well as liquid.

Thirty minutes after feeding. The lumina of both mid-gut and diverticula were filled with a mixture of solid and liquid. The solid material was not wholly digestible because fragments of arthropod exoskeleton were present, although the peripheral region of the food-mass was wholly liquid. The hind-gut lumen contained fragmented food and a few digestive cell faeces which lay centrally; it was assumed that the latter were from a feed previous to the experimental one.

Two hours after feeding. The lumina of the diverticula contained only liquid food ('*Brei*'), whereas the mid-gut contents consisted of a central mass of solid with a wide peripheral band of *Brei*. The hind-gut lumen contained solid particles, most of which were indigestible exoskeleton, encircled by a few hind-gut cell apices.

Six hours after feeding. The diverticular lumina contained not only *Brei* but also digestive cell apices (fig. 4, A), and the mid-gut lumen had solid particles and a wide peripheral band of *Brei* plus a few digestive cell apices. In the hind-gut lumen there was some *Brei* in addition to the solid particles and hind-gut cell apices.

Twelve hours after feeding. The diverticular lumina were filled with *Brei*, which had a finely granulated rather than wholly liquid appearance. Numerous digestive cell apices and a few digestive cell faeces were present. The mid-gut lumen contained a central mass of solid particles surrounded by granular *Brei* and numerous digestive cell apices. In the hind-gut the solid particles plus

granular *Brei* were surrounded by hind-gut cell apices which formed a membrane around the hind-gut contents.

Eighteen hours after feeding. Only a few digestive cell apices were interspersed with the granular *Brei* in the diverticular lumina, but numerous digestive cell faeces were present. In the mid-gut lumen solid particles

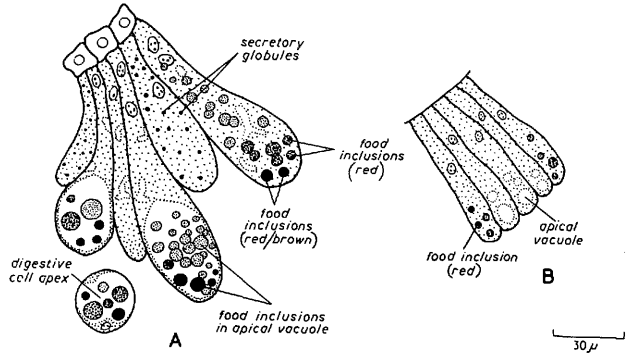

FIG. 4. A, two secretory and 4 diverticular digestive cells; one of the latter has shed its apex. B, mid-gut cells. Both 6 h after feeding. Susa: haemalum / acid fuchsin / metanil yellow / light green.

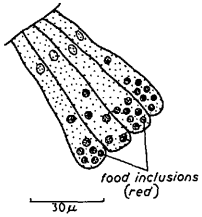

FIG. 5. Mid-gut cells 12 h after feeding. Susa: haemalum / acid fuchsin / metanil yellow / light green.

were no longer obvious, and the granular *Brei* plus the few digestive cell apices (fig. 6, A) and faeces which were present had formed into a pellet-like mass. The hind-gut lumen contained in addition to solid particles a few centrally placed digestive cell apices and faeces, the whole surrounded by a membrane formed by the hind-gut cell apices.

One day after feeding. Very few digestive cell apices and a few digestive cell faeces mixed with *Brei* were present in the diverticular lumina. The mid-gut lumen contained digestive cell apices and faeces, the former in a central and the latter in a peripheral position relative to the granular *Brei*. In the hind-gut lumen were digestive cell apices and faeces as well as granular *Brei*.

Two days after feeding. The diverticular lumina still contained granular *Brei* but no digestive cell apices and only a few digestive cell faeces (fig. 7). The mid-gut cell lumen did contain digestive cell faeces, centrally placed in

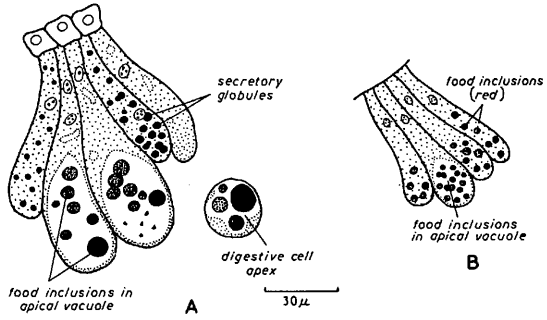

FIG. 6. A, two secretory and 3 diverticular digestive cells; one of the latter has shed its apex. B, mid-gut cells. Both 18 h after feeding. Susa: haemalum / acid fuchsin / metanil yellow / light green.

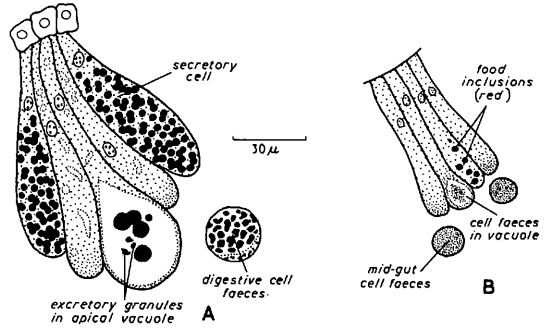

FIG. 7. A, two secretory and 3 diverticular digestive cells. Two of the latter have shed their cell faeces. B, mid-gut cells, two of which have shed their cell faeces. Both 48 h after feeding. Susa: haemalum / acid fuchsin / metanil yellow / light green.

the granular *Brei*, and mid-gut cell faeces in a peripheral position. The faecal pellet in the hind-gut was similar to that described for 24 h after feeding, but less indigestible food material was present.

Four days after feeding. Little or no granular *Brei* was present in the diverticular lumina and only a few digestive cell faeces. The contents of the mid-gut lumen were as described for 2 days after feeding. The hind-gut contained mid-gut cell faeces in addition to digestive cell faeces, but no indigestible solid particles were present.

Six days after feeding. The contents of the diverticular lumina were as described for 4 days and the mid-gut lumen no longer contained granular *Brei*, only numerous mid-gut cell faeces. The hind-gut lumen was also comparatively empty and contained a little granular *Brei*, a few digestive cell faeces, and many mid-gut cell faeces.

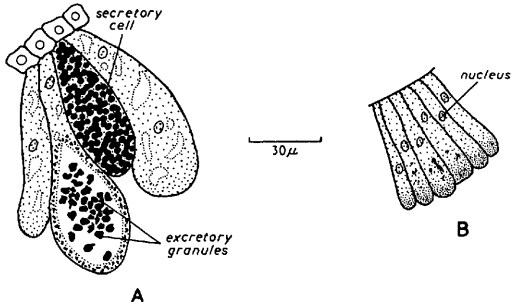

FIG. 8, A, one secretory and 3 diverticular digestive cells, one of which still contains excretory granules. B, mid-gut cells. Both 6 days after feeding. Susa: haemalum / acid fuchsin / metanil yellow / light green.

DISCUSSION

The examination of the gross morphology of the gut of *M. morio* agrees in general with the description given by Kästner (1933) for the closely related *O. parietinus* and *P. opilio*, but it is evident that 4 and not 3 pairs of lateral diverticular openings occur in the anterior mid-gut.

In agreement with Frank (1937), both particulate and liquid food was taken into the lumina of the diverticula and mid-gut of *M. morio*, and within 30 min after feeding the diverticular secretory cell contents, which are visible in starved animals, had disappeared, presumably emptied into the diverticula and mid-gut lumina. This establishes that one of the cell types in the diverticula is secretory as described by Millot (1926) for spiders, Pavlovsky and Zarin (1926) for scorpions, and Frank (1937) for harvest-spiders, and not excretory as found in pseudoscorpions by Gilbert (1952). Gilbert's main reason for assigning an excretory rather than secretory role to the second type of diverticular cell in pseudoscorpions (even though the products of the cells were in some respects similar to those of the secretory cells of spiders) was that there seemed to be no direct relationship between the activity of the digestive cells and that of the 'excretory' cells. Further, Gilbert (1952) states that Malpighian tubules are absent in pseudoscorpions, whereas they are present in spiders, scorpions, and harvest-spiders. This suggests that the lack in pseudoscorpions may be associated with the presence of excretory rather than secretory cells in the diverticula. However, it is possible that Gilbert (1952) was mistaken in his

conclusions about the function of the diverticular cells in the Chelonethi in that harvest-spiders, like pseudoscorpions, do not possess Malpighian tubules (Berland, 1949) and yet *M. morio* still has diverticular secretory cells.

Unlike spiders (Millot, 1926), the globules of the secretory cells in *M. morio* were not visible as morular masses in the gut lumina. At the same time the periphery of the food-mass within the gut was in a liquid state (*Brei*), indicating that extracellular digestion was taking place, a feature that both Kästner (1935) and Frank (1937) noted. The *Brei* was taken up by the diverticular digestive cells and formed protein globules of varied size. To judge by the changing staining reaction of the globules over a period of 24 h, they were undergoing further digestion. The colour changes observed in the diverticular digestive cell inclusions agreed closely with those reported in the Chelonethi by Gilbert (1952).

Six hours after feeding, digestive cell apices were cut off into the diverticular lumina. This compares with Frank's (1937) findings that cell apices cut off as early as 8 h after feeding differed from the later 'cell faeces' in that they contained not only excreta but also globules. Gilbert (1952) found digestive cell apices cut off in the Chelonethi quite soon after feeding but the contents had broken down into 'cell faeces' without the cellular inclusions having gone through the normal acid-fuchsinophil stage. At this time, in *M. morio*, small globules first appeared in the diverticular secretory cells. It was also at this stage, when digestive cell apices entered the mid-gut lumen, that small cellular inclusions plus apical vacuoles appeared in the mid-gut cells. The presence of vacuoles in the mid-gut cells (not noted by earlier workers) 6 h after feeding cannot be explained but may have some connexion with the change in appearance of the *Brei* from a liquid to a granular stage, as observed 12 h after feeding when the vacuoles had disappeared.

The number of mid-gut cell inclusions increased as the number of digestive cell apices in the mid-gut decreased, and it is assumed that the mid-gut cells took up the partially digested material of the digestive cell apices. Kästner (1935) believed that the digestive cell apices were probably stored in the gut. However, according to Frank (1937), the digestive cell apices on reaching the mid-gut lumen lose their contained globules, and he suggested that they were absorbed by the mid-gut cells. He noted also that small globules of unknown origin and development, but presumably metabolic products, were present in the gut cells in considerable numbers during active digestion. The relationship between an increased number of mid-gut cell inclusions and the disappearance of digestive cell apices was not realized by Frank (1937), and it was stated that no mid-gut cell faeces were produced. It is evident from the present work that mid-gut cell faeces were produced about 24 h after feeding and that the mid-gut cells complete the digestion of the partially denatured protein of the digestive cell apices. This procedure differs from that in the Chelonethi, where, according to Gilbert (1952), the digestive cell apices/faeces remained in the gut lumen as faeces, but the 'excretory cell' apices were taken up in the syncytial epithelium of the post-diverticular mid-gut and there converted into

amorphous material accompanied by the secretion of a similar material into the lumen.

While mid-gut cell digestion was taking place in *M. morio*, further activity occurred in the diverticular digestive cells, and digestive cell faeces, equivalent to the cell faeces of both Frank (1937) and Gilbert (1952), were produced. The digestive and mid-gut cell faeces became mixed with the indigestible material of the mid-gut contents and were passed into the hind-gut.

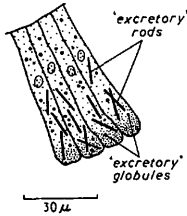

FIG. 9. Hind-gut cells 6 days after feeding. Susa: Heidenhain's iron haematoxylin.

In the hind-gut a membrane was formed round the faecal material by the hind-gut cell apices as described by Frank (1937). The formation of the first faecal membrane at 12 to 18 h after feeding agrees with Phillipson's (1960) findings that the first faecal pellet from a given food is voided $15{\cdot}5 \pm 1{\cdot}5$ h after feeding in males and $19{\cdot}7 \pm 3{\cdot}2$ h in females.

The presence of rod-shaped structures and globules during starvation in most of the mid-gut cells in sections stained with Heidenhain's haematoxylin (fig. 9) is not understood. Frank (1937) noted the rods in the oesophageal cells only and suggested that their function might be either skeletal or food storage. Their presence in the mid-gut cells during starvation and at no other time does not support either theory but suggests a possible excretory function.

I should like to thank Prof. J. B. Cragg for his help in the writing of this paper.

REFERENCES

BERLAND, L., 1949. In Grassé, 1949, **6**, 761.
FRANK, H., 1937. Z. Morph. Ökol. Tiere, **33**, 151.
GILBERT, O., 1952. Quart. J. micr. Sci., **93**, 31.
GRASSÉ, P.-P., 1949. Various authors in chapter on Classe des Arachnides, in *Traité de Zoologie*, **6**, 263. Paris (Masson).
KÄSTNER, A., 1933. Z. Morph. Ökol. Tiere, **27**, 587.
——, 1934. Zool. Anz., **106**, 257.
——, 1935. In Kukenthal & Krumbach, *Handbuch der Zoologie*, Bd 3(2), Lief 9(2), 300. Berlin (de Gruyter).
MILLOT, J., 1926. Bull. biol. Fr. Belg., Supplement **8**, 1.
PAVLOVSKY, E. N. and E. J. ZARIN, 1926. Quart. J. micr. Sci., **70**, 221.
PHILLIPSON, J., 1960. J. Anim. Ecol., **29**, 35.