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ADAPTIVE FEATURES OF GUT STRUCTURE AND DIGESTIVE PHYSIOLOGY IN THE TERRESTRIAL ISOPOD *PHILOSCIA MUSCORUM* (SCOPOLI) 1763.

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The alimentary canal of isopod crustaceans is comparable to that of most other Arthropoda in that three basic regions can be recognized. These are the foregut, derived from the ectodermal stomodaeum and consisting of an oesophagus and proventriculus, the endodermal midgut, and an ectodermal hindgut derived from the proctodaeum (Goodrich, 1939). The midgut, though, is unusual in that it is virtually restricted to four or six simple caeca which arise at the junction of the fore- and hindgut. Controversy over whether any other part of the alimentary canal is of endodermal origin, and therefore to be regarded as midgut, has been resolved by Holdich (1973) who concludes from a comprehensive review of the literature that the discontinuity between the chitinous intimas of the fore- and hindgut described by Goodrich (1939), underlain in some species by a band of endoderm two to three cells wide, is the only remnant of a true midgut in isopods, other than the caeca.

The terrestrial isopods show further modifications of the alimentary canal in that the hindgut is subdivided into an anterior somewhat expanded region, which bears along its length a dorsal typhlosole, a median papillate region, a muscular sphincter, and a short posterior rectum (Sutton, 1972).

The histology and ultrastructure of the isopod gut is known from a considerable number of studies, summarized in Jones, Babbage and King (1969), Schmitz and Schultz (1969), Holdich and Ratcliffe (1970), Clifford and Witkus (1971) and Alikhan (1972). The caeca have received particular attention and most authors agree that they are the source of endogenous digestive enzymes which act on the food within the caecal lumen, the proventriculus, or the hindgut. The caeca are also concerned with absorption of digested food and in some instances are believed to carry on digestion intracellularly.

Little is known, though, of the functional significance of the reduction of the midgut to four or six caeca and, in terrestrial species, of the subdivision of the hindgut and the development of a typhlosole. It would seem likely, however, that the latter modifications are related in some way to the terrestrial habit, probably as adaptations to the type of food available. The terrestrial isopods have a large proportion of cellulose in their diet, feeding on moist vegetable litter and often supplementing this by consuming their own faeces (Wieser, 1966; Sutton, 1972). They show strong preferences for partly decomposed litter rich in the microorganisms which effect its decomposition (Edwards, 1974; Hassall, 1975a) and their gut modifications may well be adaptive features facilitating efficient utilization of this food, possibly by allowing the isopods to make extensive use of the cellulose-degrading properties of the litter microflora. Endogenous cellulases are not com-

mon in animals and herbivores are often dependent on microorganisms for cellulose digestion (Buchner, 1965). In insects these are usually symbiotic inhabitants of the gut (Lasker, 1959) but in isopods, where only a few species have been examined for cellulases, the situation is less clear. Ray and Julian (1952) and Ray (1959a) found no evidence of a microflora in the gut of the marine wood-borer *Limnoria lignorum* and concluded that the cellulases present were secreted by the cells of the gut caeca. This conclusion was also reached by Hartenstein (1964) for the terrestrial *Oniscus asellus,* but no firm evidence for actual secretion from the caecal cells was presented for either species.

The present study has been undertaken to assess the functional significance of the various modifications in mid- and hindgut structure seen in terrestrial isopods, to establish the general pattern of their digestive physiology and to ascertain the origin of the cellulases acting in their gut. The species selected for the study was *Philoscia muscorum* (Scopoli) 1763, an isopod known to be a macrodecomposer in British dune grassland ecosystems and whose diet, feeding mechanisms and foregut functional morphology have already been described (Hassall, 1975b).

Materials and Methods

Philoscia muscorum was collected from a dune grassland at Spurn Head, East Yorkshire and maintained under semi-natural conditions in large fiberglass tanks containing sand, turf and leaf litter from the natural habitat.

Specimens selected for study were starved for five days in 15 cm diameter plastic pots, on 2mm mesh nylon gauze suspended 2 cm above a moist pad of plaster of Paris. Faeces fell through the gauze as they were produced and could not be ingested. This procedure cleared the gut of remnants of previous meals, encouraged subsequent ingestion of test meals and facilitated section cutting by removing ligneous and siliceous particles derived from the natural diet.

The structure of the mid- and hindgut and the course of digestion were studied by histological, histochemical and substrate film methods, using alimentary canals removed either directly from the starved animals or at intervals after the latter had been observed to ingest test meals of boiled potato or well decayed leaf litter.

Histological methods

Whole animals, and intact alimentary systems removed by dissection, were fixed for twenty four hours in Bouin's fluid. Penetration of fixative into the whole animals was facilitated by removing the lateral regions of the pereionites and pleonites. Serial sections were stained by Ehrlich's haematoxylin and eosin, Mallory's triple stain, Feulgen's reaction for D.N.A., or the periodic acid-Schiff reaction. The progressive digestion of starch in potato-fed specimens was followed in whole mounts and sections by staining with Lugol's iodine or periodic acid-Schiff.

Histochemical methods

Specimens were immobilized by cooling in the freezing compartment of a refrigerator. They were then flooded with cold saline [0.34 M sodium chloride as used by Schmitz and Schultz (1969) for *Armadillidium*] and the gut rapidly dis-

sected out. Serial sections were obtained by fixing for twelve hours at 1° C in 10% formalin buffered to pH 7.0, dehydrating in graded cold acetones, clearing in xylene and impregnating *in vacuo* in paraffin melting at 45° C (Jennings, 1962). The sections were examined for enzymic activity using: a) the indoxyl acetate method for non-specific esterase (Holt, 1958) either alone or with various activators and inhibitors (Pearse, 1961); b) the L-leucyl β naphthylamide hydrochloride method for arylamidases (Burstone and Folk, 1956); and c) the naphthyl AS-BI phosphate methods for acid and alkaline phosphatases (Burstone, 1958).

Enzymic activity was also studied by applying the above methods to intact alimentary canals, removed from immobilized starved and fed individuals, fixed by immersion for twelve hours in absolute acetone at 1° C.

Controls for the histochemical investigations consisted of heat inactivated sections, incubation of normal sections in media lacking the specific substrate and incubation in the normal media of sections of appropriate mammalian tissues.

The techniques for acid and alkaline phosphatases indicated some possible sites of absorption. Confirmation of these was sought by using the iron saccharate method of Yonge (1926), feeding starved individuals on boiled potato mixed with saccharated ferrous carbonate. These were fixed at progressive intervals in equal parts of Bouin's fixative and 5% ammonium sulfide in 95% alcohol, and examined for absorbed iron after treating sections with 10% aqueous potassium ferrocyanide followed by 0.1 N hydrochloric acid. Controls consisted of starved individuals fed on potato only, to detect endogenous iron; and of intact alimentary canals removed from starved individuals, filled by injection with saccharated ferrous carbonate and immediately fixed. This demonstrated diffusion artifacts arising during fixation and subsequent procedures.

Substrate film methods

The occurrence and distribution of cellulases were studied using the substrate film method devised by Sumner (1968) in which the tissue or section to be examined is placed beneath a thin film of carboxymethyl cellulose ("Cellofas B 10", I.C.I. Ltd., England). Any cellulases present hydrolyze the film adjacent to their position in the specimen, the hydrolyzed areas subsequently remaining clear when the film is stained with 0.05% toluidine blue. Diffusion of cellulases was reduced by prior fixation of materials in formaldehyde-calcium (Baker, 1960) for one hour at 4° C.

The materials tested were intact alimentary canals, and frozen sections 15 μm thick through various regions of the canal, from individuals fixed after starvation and after meals of either the natural food of decomposing leaf litter or boiled potato. Samples of boiled potato, the natural food and of the faeces were also tested.

Controls for the substrate film tests consisted of heat inactivated specimens, and squashes of the digestive gland of *Helix aspersa* fixed and treated in the same manner as the other materials.

Observations and Results

The alimentary canal in *Philoscia muscorum* (Fig. 1) has the form characteristic of terrestrial isopods, comprising an oesophagus and proventriculus (foregut),

four tapering caeca (midgut) which arise from a small common caecal chamber situated ventrally at the junction of the proventriculus and hindgut, and a hindgut subdivided into four distinct regions.

The mouthparts, feeding mechanism and functional morphology of the foregut have been described elsewhere (Hassall, 1975b). Briefly, decomposing leaf litter is fragmented by the mandibles, pushed into the mouth by the other mouthparts and passed down the oesophagus into the proventriculus. The proventriculus presses the food, squeezing out liquids and channelling them ventrally into the midgut caeca while solids are directed into the anterior chamber of the hindgut where they may remain for up to seven days. The walls of the proventriculus bear internal lamellae and when the organ is empty it can be moved by its extrinsic musculature in such a way that secretions from the caeca can be directed

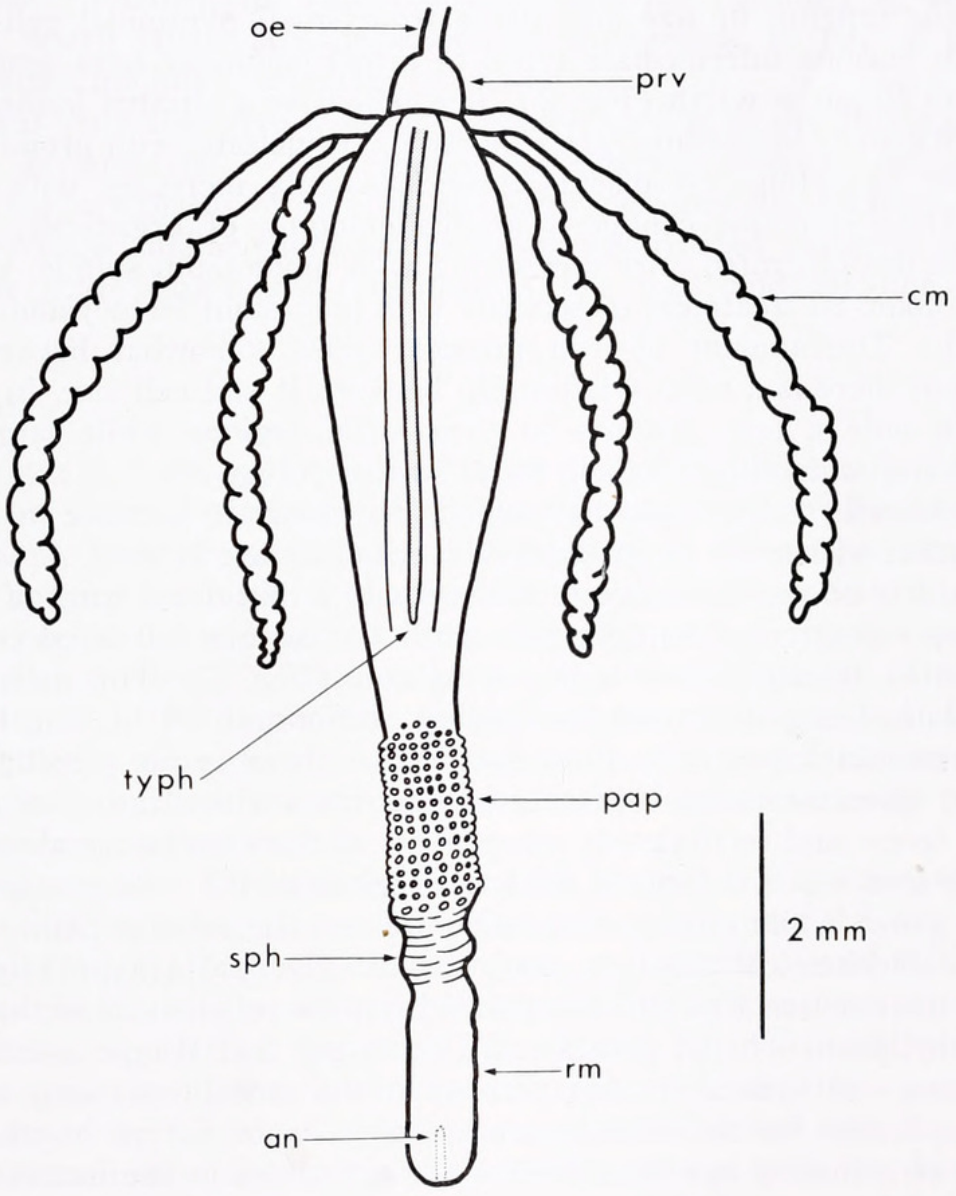


FIGURE 1. Alimentary canal of *Philoscia muscorum* from the dorsal aspect. The origins of the caeca from the common ventral chamber beneath the proventriculus are not shown: an represents position of postero-ventral anal slit; cm, caecum; oe, oesophagus; pap, papillate region of hindgut; prv, proventriculus; rm, rectum; sph, sphincter; typh, typhlosole.

upwards and into the ends of the two channels formed from the upturned lateral projections of the typhlosole which runs along the inner dorsal surface of the anterior portion of the hindgut.

The caeca (midgut)

In adult *P. muscorum* the caeca (Figs. 1 and 2) are 4.0–5.0 mm in length and 0.5–0.8 mm in diameter. They curve upwards and backwards from their origin below the proventriculus and lie freely in the body cavity. Externally they appear as spiral structures due to the presence of thin sheets of muscle which run obliquely around them. The muscles are not differentiated into circular and longitudinal components, but their oblique path presumably compensates for this in that it allows them to change both the length and diameter of the caeca and so draw in, or expel, food materials and digestive secretions.

The principal component of the caecal wall is a monolayered endothelium composed of cells ranging in size and shape from small pyramidal cells, 20–30 μm tall, through various intermediate types to broad columnar ones reaching 70 μm in height and 30 μm in width (Fig. 2). The cells have a striated border 0.5–0.1 μm in depth, are usually binucleate, rarely mono- or trinucleate, with prominent nucleoli and granular basophilic cytoplasm whose basophila increases with the size of the cell. The cytoplasm contains variable amounts of organically bound iron which can be demonstrated after unmasking with ammonium sulfide, when it gives a deep blue color on treatment of sections with potassium ferrocyanide and hydrochloric acid. The amount of iron present varies somewhat between similarly sized cells but there is a clear relationship between it and cell size, in that smaller cells contain only a few granules in their basal regions while larger ones are loaded with iron almost up to their distal borders (Fig. 2).

The caecal cells contain esterases which, like the iron, increase in both amount and distribution within the cytoplasm as the cells increase in size. Small pyramidal cells show either no reaction for esterases or only a thin distal band of activity, but as they grow the esterase zone deepens until the mature full sized cell shows an intense reaction through most of its cytoplasm (Fig. 3). The esterase reaction is thermolabile, being destroyed by holding sections at 90° C for five minutes, while the reaction for iron is thermostable, so there is no possibility that the reaction for esterases is an artifact resulting from visualization of intracellular iron by the ferro- and ferricyanide components of the esterase incubation medium. Further, the iron deposits form in the basal regions of the cells and spread distally as the cells grow, while esterase formation follows the reverse path.

Standard biochemical practice recognizes A-, B- and C-type esterases. The intensity of the reaction was not diminished by pre-incubation of sections in 10^{-5} M E-600 (diethyl-*p*-nitrophenyl phosphate), indicating that B-type esterases such as cholinesterases and lipases are not present in the caecal secretions and that the enzymes responsible for the reaction are probably A- or C-type esterases, or both. The results of including specific inhibitors or activators in the incubation medium after pre-incubation in E-600 suggested that, in fact, both types are present (Table I). The reaction was completely inhibited by 10^{-5} M silver nitrate (as are all A- and C-esterases) but activated by 10^{-3} M cysteine which is a characteristic of A-esterases. These, though, are inhibited by 10^{-4} M sodium *p*-chloromercuri-

TABLE I

Characterization of the E-600 resistant caecal esterase reaction in P. muscorum†

Treatment	Effect	Inference
10^{-2} M $AgNO_3$	complete inhibition	A- or C-esterases present
10^{-3} M cysteine	activation	A-esterases present
10^{-4} M PCMB*	activation	C-esterases present
10^{-3} M $Pb(NO_3)_2$	no inhibition	C-esterases present
10^{-2} M βPPA**	no inhibition	A-esterases present

† following the classification of Pearse (1961).
* sodium *p*-chloromercuribenzoate.
** β-phenylpropionic acid.

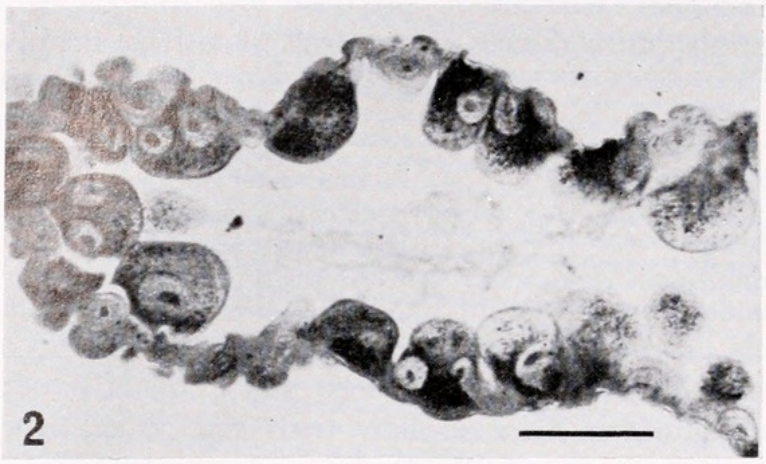
benzoate while the caecal esterases were activated by this compound, a characteristic of C-esterases. Further, the caecal reaction was not inhibited by either 10^{-3} M lead nitrate, which causes 50% inhibition of A-esterases, or 10^{-2} M β-phenylpropionic acid which inhibits C-esterases.

The caecal cells also give a slight positive reaction to the Burstone and Folk method for arylamidases. This, unlike the esterase activity, occurs uniformly throughout the cytoplasm of cells of all sizes and does not increase as the cells grow. They show similar slight but uniformly distributed reactions to Burstone's methods for acid phosphatase (incubation pH 5.2) and alkaline phosphatase (pH 8.3).

The hindgut

The hindgut in *P. muscorum* is the longest part of the alimentary canal (Fig. 1), running almost the entire length of the body and being 9–10 mm long and 1–1.5 mm broad anteriorly in adult specimens. It consists of an outer muscular layer, made up of outer longitudinal and inner circular components, a monolayered epithelium and a thin periodic acid-Schiff positive chitinous intima closely applied to the distal epithelial surface. Variations in the relative thicknesses of the muscular layer, the degree of folding of the epithelium and the shape and disposition of the epithelial cells allow four distinct regions to be recognized, morphologically and histologically. These are termed here the typhlosole, externally papillate, and sphincter regions, and the rectum.

Typhlosole region. The anterior half of the hindgut is characterized by a dorsal typhlosole which runs from its anterior end to a point about 0.8 mm short of the next region. The typhlosole is formed from an internal dorsal fold in the epithelium which has lateral expansions curving dorsally to form two parallel channels along the dorsal surface of the gut (Figs. 4 and 5). The epithelium forming the typhlosole is not differentiated into cell types and, like the epithelium lining the remainder of the anterior hindgut, consists of columnar cells 60–35 μm wide, with single dense nuclei, lightly basophilic cytoplasm and with the chitinous intima closely molded to their distal surfaces which protrude somewhat into the gut lumen (Fig. 6). They show no reaction for iron, esterases or arylamidases. All the cells, though, apart from those lining the channels of the typhlosole, show a thin distal zone of both acid and alkaline phosphatase activities (Fig. 5).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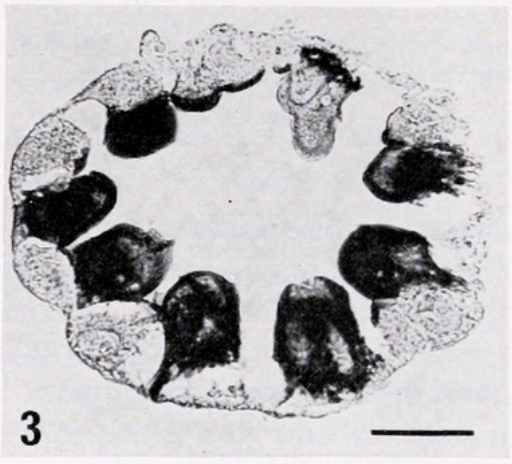
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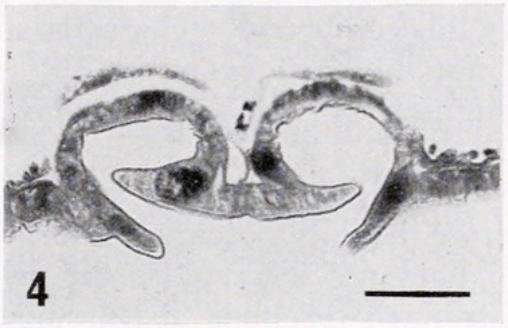
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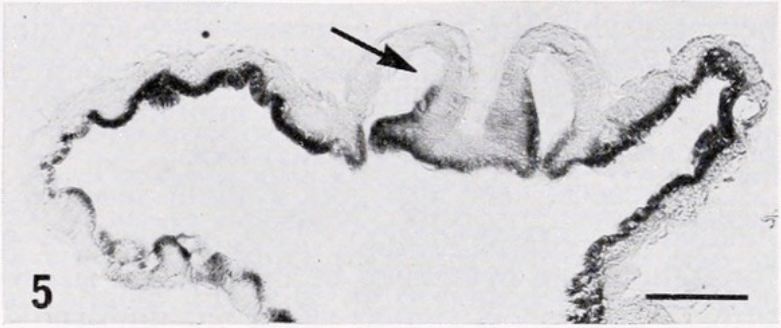
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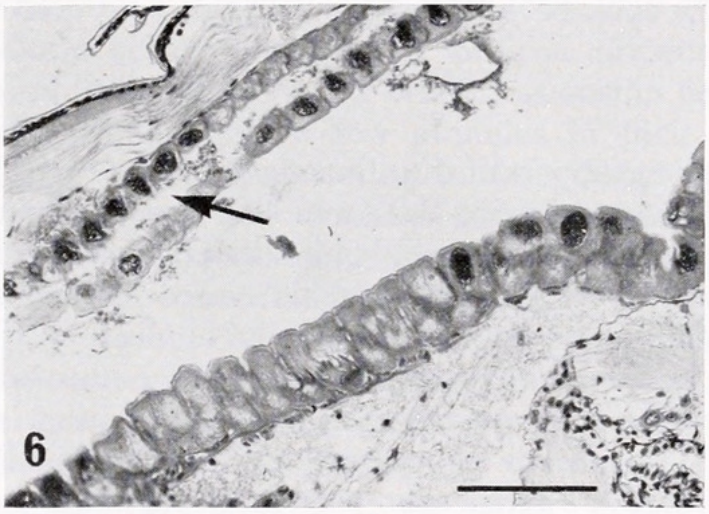
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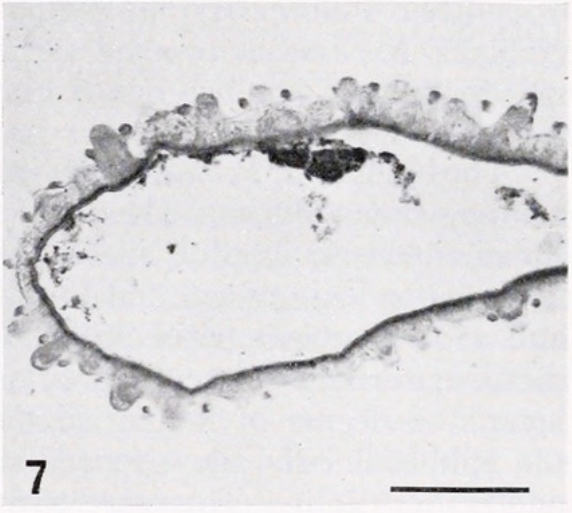
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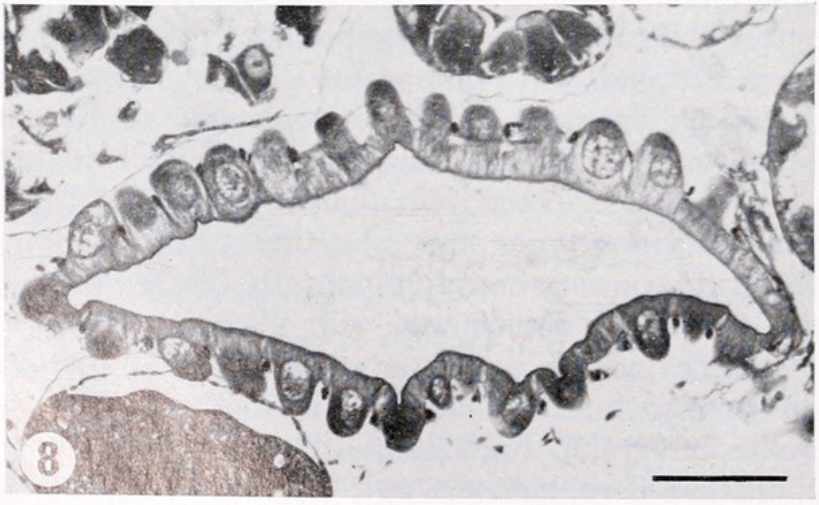
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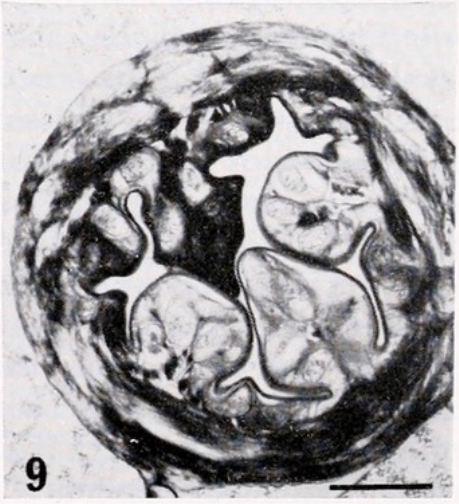
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The muscle layers of the gut wall do not enter the typhlosole. Both layers are equally developed and form a thin uniform envelope around the epithelial layer.

The externally papillate region. The second portion of the hindgut is 1.5–1.8 mm in length and externally has a characteristic papillate appearance (Fig. 1). The "papillae" are, in fact, the constituent cells of the epithelium whose basal regions project outwards into the haemocoel (Figs. 7 and 8). The cells are all of the same type, 60–70 μm tall and 35–40 μm broad, each with a large prominent nucleus and basophilic cytoplasm. The basal regions projecting into the haemocoel contain large amounts of organically bound iron, comparable to those occurring in the caecal cells, but no parts of the cells show any reactions for esterases or arylamidase. There is, however, a distal zone of acid and alkaline phosphatase activity, 2–4 μm deep, and a general but weaker reaction for acid phosphatase is given by the rest of the cytoplasm (Fig. 7).

The circular muscles show no modification in this region of the hindgut, but the longitudinal muscles become separated into distinct blocks which lie between the protruding bases of the cells (Figs. 7 and 8).

The sphincter region. The papillate region is separated from the rectum by a portion of hindgut 0.6–0.7 mm long in which the band of circular muscles in the gut wall increases in thickness from the 2–3 μm characteristic of the rest of the hindgut to 90–100 μm (Fig. 9). The longitudinal muscles remain unchanged but the epithelial layer is thrown up into pear-shaped folds which interlock when the circular muscles contract and thus seal off the papillate region from the rectum.

The muscular layers show a uniform reaction for acid phosphatase but the epithelial layer shows no enzymic reactions and does not contain iron.

The rectum. The terminal portion of the hindgut is 1–1.2 mm long and opens to the exterior at the slit-like anus. The muscular layers are unmodified; the epithelium is folded but not to the same extent as in the sphincter region and the

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FIGURE 2. *P. muscorum;* longitudinal section of a caecum showing differential distribution of iron (black) in the epithelial cells; ammonium sulfide, potassium ferrocyanide and hydrochloric acid technique; scale = 100 μm.

FIGURE 3. Transverse section of a caecum showing esterase activity (black) in the epithelial cells. Section is treated by Holt's method for non-specific esterase; scale = 50 μm.

FIGURE 4. Transverse section through the median dorsal region of the anterior hindgut showing the typhlosole. Feulgen and fast green; scale = 200 μm.

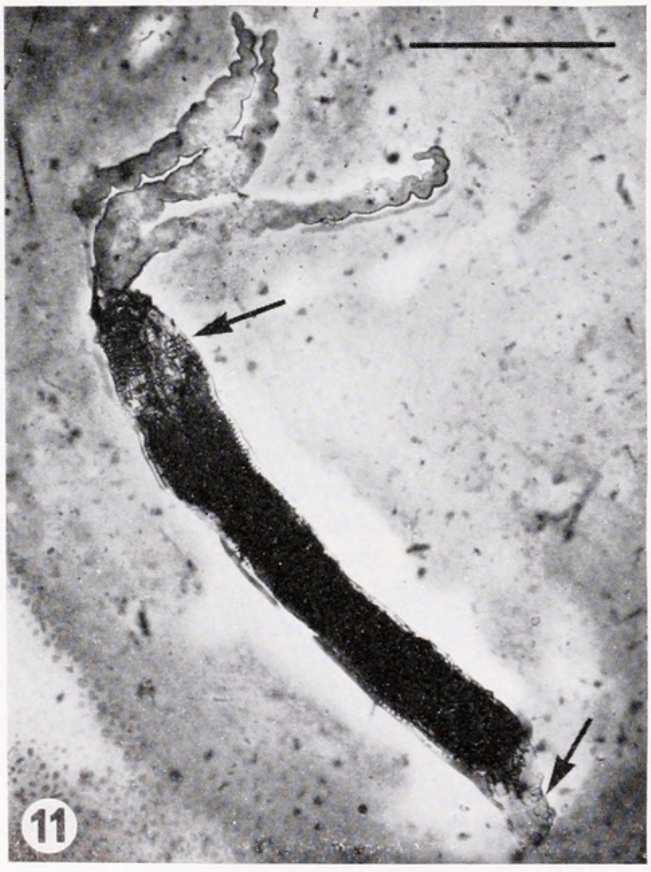
FIGURE 5. Transverse section through the anterior hindgut showing alkaline phosphatase activity (black) in the distal regions of all epithelial cells except those lining the typhlosole channels (arrowed). Section is treated by Burstone's method for alkaline phosphatase; scale = 200 μm.

FIGURE 6. Longitudinal section through the anterior hindgut showing the ventral epithelium (cut obliquely) and one of the channels (arrowed) formed by the typhlosole. Section is stained with haematoxylin and eosin; scale = 200 μm.

FIGURE 7. Transverse section of the papillate region of the hindgut showing a diffuse reaction for acid phosphatase throughout the cytoplasm and a stronger distal band. Section is treated by Burstone's method for acid phosphatase; scale = 250 μm.

FIGURE 8. Transverse section of the papillate region showing the epithelial cells protruding outwards into the haemocoel, with longitudinal muscle blocks lying between them. Section is stained with Mallory's triple stain; scale = 150 μm.

FIGURE 9. Transverse section of the sphincter region of the hindgut showing the increase in thickness of the circular muscles and the pear-shaped folds of the epithelium. Section is stained with Mallory's triple stain; scale = 100 μm.



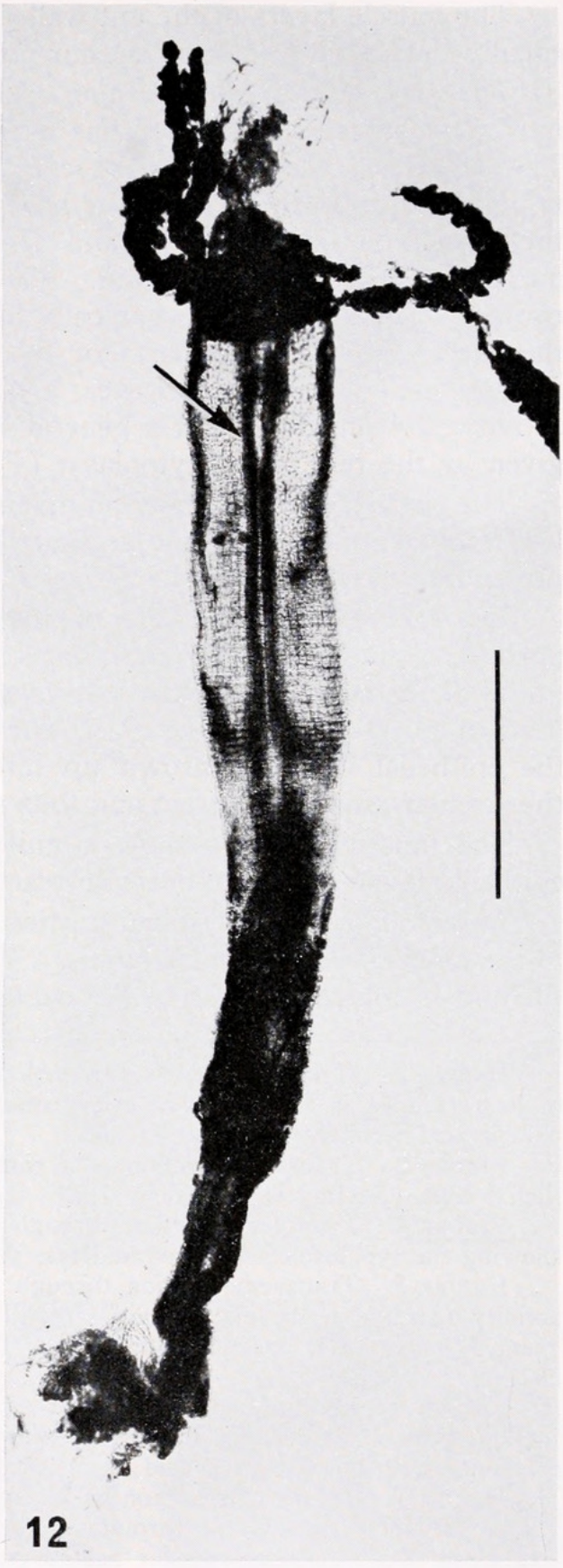


FIGURE 10. *P. muscorum;* alimentary canal fixed after a meal of decomposing leaf litter, covered with a thin film of carboxymethyl cellulose, incubated for one hour and subsequently stained in toluidine blue. Parts of the film hydrolyzed by cellulases diffusing from the specimen appear white, while unchanged areas appear grey; scale = 2 mm.

FIGURE 11. As Figure 10 but showing reduced cellulase activity in those regions of the hindgut (anterior hindgut and rectum, arrowed) which contain little or no food. Areas of the

chitinous intima is thicker and stains more heavily with periodic acid-Schiff. The epithelial cells contain both acid and alkaline phosphatases uniformly distributed throughout their cytoplasm, but give no reaction for esterases, arylamidases or iron.

Cellulases

Intact alimentary canals from starved animals and from individuals fed for at least 5 days on boiled potato showed no reaction when tested by Sumner's substrate film method, indicating that there are neither endogenous cellulases nor a permanent symbiotic microflora producing cellulases in *P. muscorum*. In marked contrast, though, very strong cellulase activity was found in the fore- and hindguts of specimens which had ingested the natural food of decaying leaf litter, but the caeca consistently gave negative results (Figs. 10 and 11). Confirmation that the cellulases responsible for digestion of the substrate film were already present in the food, before ingestion, came from testing uningested food, which showed a positive reaction, and from examination of alimentary canals from isopods which had taken only small meals. Such specimens had regions of the gut which contained little or no food (*e.g.* the anterior portion of the typhlosole region of the hindgut and the rectum in the specimen shown in Fig. 11) and cellulase activity was either reduced or absent in these areas.

The incubation time in all cases was standardized at one hour to allow some comparison of the relative degrees of activity in the food before and after ingestion. So far as could be judged the food showed greater cellulase activity after being confined in the hindgut for 6–12 hours, indicating that there is perhaps some factor operating there which favors growth or enzyme production by the microorganisms. Alternatively the increased activity may have been due simply to closer packing of the food materials, resulting in increased numbers of microorganisms per unit volume as compared with the loose uncompressed state of the food before ingestion. Freshly passed faeces, however, also showed higher levels of cellulase activity but these decreased with time until 6–7 days later when they were the same as those seen in samples of the natural food.

Heat inactivated preparations of the food, and of alimentary canals from fed individuals, showed no cellulase activity.

A single starved isopod was induced to ingest a freshly prepared liquid paste of the carboxymethyl cellulose used in the substrate film investigations. It was fixed 12 hours after feeding in the acid ethanol used during preparation of the substrate films and subsequently stained with toluidine blue. Small amounts of unchanged carboxymethyl cellulose were seen in the lumen of the caeca, providing confirmatory evidence for the absence of endogenous cellulases from the caeca and for the entry on at least some occasions of fluid materials into these structures.

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film adjacent to the caeca show no hydrolysis; the narrow lighter zone adjacent to the lower caecum is the result of refraction of light by the cellulose film during photography and in the original preparation appeared as blue as the remainder of the unchanged film; scale = 2 mm.

FIGURE 12. Alimentary canal fixed 24 hours after a meal of boiled potato, showing intense esterase activity (black) in the caeca, proventriculus, typhlosole channels (arrowed) and papillate region. Section is treated by Holt's method for nonspecific esterase; scale = 2 mm.

Digestion and absorption

The course of digestion was followed in sections of alimentary canals from isopods fixed at progressive intervals up to 7 days after they had fed on boiled potato devoid of intrinsic enzymes. During feeding the proventriculus rapidly filled with potato and then gradually emptied over the next 5–10 minutes as the contents were passed onwards into the hindgut. The proventriculus then filled again and the process continued until the anterior hindgut and the papillate region were packed. At this point the isopods stopped feeding and the proventriculus remained empty.

The food developed a slight but distinct esterase reaction as it passed into the the hindgut. The reaction showed the same responses to activators and inhibitors as those given by the esterases seen in the caecal cells and it is concluded therefore, that the caeca discharge a small amount of enzymes, exemplified by esterases, onto the food as it passes through the proventriculus. Support for this came from the observation that the esterase reaction in the caecal cells declined in intensity during feeding, but then gradually returned to normal over the next six hours.

The food lying in the hindgut continued to show a low level of esterase activity until 20–24 hours after ingestion. About this time the contents of the papillate region showed a marked increase in esterase activity and this coincided with the appearance of esterases in the two channels formed by the lateral expansions of the typhlosole (Fig. 12). Esterases also appeared in the proventriculus so that there was a continuous band of enzymatic activity between the caeca and papillate region. Sections of the typhlosole showed that the enzymes were not originating in the endothelial layer and it is concluded that they are secreted by the caecal cells, poured into the proventriculus and directed by this into the two channels of the typhlosole. They thus bypass the anterior hindgut and are discharged from the posterior end of the typhlosole onto the food lying in the papillate region.

During the 3–4 days after this increase in esterase activity material moved slowly from the papillate region into the rectum and was then voided as faeces. It was replaced by material from the anterior portion of the hindgut, which in turn developed increased esterase activity as it entered the papillate region, and this process continued until the hindgut was completely empty some 7 days after the observed meal. It is presumed that under natural conditions, though, the gut would rarely be empty and that there would be a slow but steady movement of material through the alimentary canal, with regular additions to it of esterases, *via* the typhlosole, in the papillate region.

The caecal cells normally show some reactions for acid and alkaline phosphatases and arylamidases but these enzymes were never demonstrated in the secretion passing along the typhlosole canals. Some slight arylamidase activity did develop, however, in food as it passed through the proventriculus and this persisted for as long as the material remained in the hindgut. The enzyme, or enzymes, responsible are presumably secreted by the caecal cells along with the esterases added to the food, soon after ingestion, in the proventriculus.

Treatment of sections of potato-fed isopods with Lugol's iodine or periodic acid-Schiff showed that there is no hydrolysis of starch in the proventriculus. A small amount of hydrolysis was seen in the typhlosole-bearing portion of the hindgut

but the largest amount was found in the papillate region. At no time, though, was there hydrolysis of the entire meal and in all specimens examined there were always considerable quantities of unchanged starch in the hindgut. The faeces, similarly, always contained much starch and it would appear that *P. muscorum* has only a low assimilation efficiency for this type of food, and, indeed, for the natural food, much of which passes through the alimentary canal apparently little changed.

The high level of esterase activity in the papillate region suggested that this is the principal site of endogenous enzyme activity and since phosphatases occur in the distal portions of the epithelial cells it was thought likely that this part of the hindgut may be the principal site of absorption of digestive products. Phosphatases occur in the epithelium of the typhlosole region but not in that lining the typhlosole channels (Fig. 5) and in the caeca, so that absorption would be expected at these sites also. Attempts were made to demonstrate absorption by using saccharated ferrous carbonate as a component of test meals, but the results had to be interpreted with caution. Control sections of both starved and potato-fed individuals showed that the epithelial cells of the caeca and papillate region normally contain substantial quantities of iron, as described earlier, so that absorption of iron or iron-containing compounds by these cells in the specimens fed saccharated ferrous carbonate could only be deduced if they showed a significant increase above this pre-existing level. Further, the controls in which the saccharated ferrous carbonate was injected mechanically into alimentary canals that were then fixed immediately and processed, showed that diffusion artifacts could arise where large amounts of the carbonate were present in the gut lumen. Despite these limitations, there was clear evidence of absorption in the caeca and in the anterior and papillate regions of the hindgut, with the epithelial cells showing slight but significant increases in iron content. The most significant result derived from these particular feeding experiments, though, was the appearance of small amounts of saccharated ferrous carbonate within the lumen of the caeca without any traces of the potato tissue which had formed the bulk of the test meal and which was present in the proventriculus and hindgut. This gave further confirmation that components of a meal can be separated by the proventriculus and that substances in solution can be diverted into the caeca.

Discussion

Three salient points emerge from this study of gut structure and function in the terrestrial isopod *Philoscia muscorum*. These are that the caeca are confirmed as the major source of endogenous digestive enzymes; that the function of the typhlosole is to conduct enzymes from the caeca to the papillate region of the hindgut and thereby allow them to bypass the anterior hindgut; and that the cellulases operating in the gut are already present in the food before ingestion and are not produced by the animal or by permanent symbiotic gut flora.

The separation of liquid components from the food by the proventriculus and their diversion into the caeca, described by Hassall (1975b) and confirmed here, presumably occurs during or very soon after ingestion since there is also flow of enzymes in the reverse direction, from the caeca, onto the solid food as this passes into the hindgut. It seems unlikely, though, that there will be much diges-

tion in the caecal lumen as solids have never been found there. The liquids derived from the natural food probably contain simple substances which require little or no treatment before absorption, and the capacity for absorption in the caeca was fairly clearly demonstrated in the saccharated ferrous carbonate feeding experiments. It may well be that the caeca are major sites of water absorption, and perhaps water storage, and that absorption of nutrients in this part of the gut is of only secondary importance.

There is no obvious differentiation of the caecal epithelium into secretory and absorptive cells; the differences in the size and shape of the constituent cells have been interpreted, in other species, as indicating the presence of at least two types of cell (Murlin, 1902; Chandy, 1938; Alikhan, 1969; Clifford and Witkus, 1971; Hryniewiecka-Szyfter, 1972) but an alternative view is that only one type is present and that the differences simply reflect different stages in its growth (Beecher-Moore, 1956; Donadey, 1969; Jones, Babbage and King, 1969; Schmitz and Schultz, 1969). The range of cell sizes, and the direct relationship between size on the one hand and basophilia and iron and esterase content on the other, seen in *P. muscorum* suggest that for this species, at least, the second interpretation is correct. It seems likely that the smaller cells are, in fact, younger cells which go through an initial absorptive phase, in which they take up material from the caecal lumen, before they mature and assume a predominantly secretory function. During this second phase the cells produce digestive enzymes, exemplified by esterases, which affect hydrolysis of food principally at sites other than the caecal lumen.

The origin and physiological role of the iron deposits in the caecal cells and, indeed, in those of the papillate region, are unknown; similar accumulations of this metal and of copper are a characteristic but as yet unexplained feature of isopod gut caeca (various authors, summarized in Wieser and Klima, 1969; Alikhan, 1972; Hryniewiecka-Szyfter, 1972).

The caecal enzymes operate at two separate points in the digestive process. They are secreted first in only small amounts onto the food as it passes through the proventriculus and remain active, albeit at a relatively low level, for the next 20–24 hours. The consistently low level indicates, incidentally, that there is no supplementary secretion of esterases from the hindgut endothelium. The second and major addition of caecal enzymes to the food occurs at the end of this period but, unlike the first and minor secretion, the enzymes are not discharged onto food in the proventriculus or anterior hindgut. Instead, they are diverted *via* the proventriculus into the typhlosole channels, bypass the anterior hindgut and are discharged onto material lying in the second, papillate, region of the hindgut.

The functional and adaptive significance of the typhlosole and of the subdivision of the hindgut into anterior and papillate regions, now becomes apparent. *P. muscorum,* in common with other terrestrial isopods possessing these features, feeds on decomposing plant materials rich in microorganisms which produce, among other enzymes, cellulases. The modification of the alimentary canal allows the isopods to exploit to the fullest the degradative capabilities of these free-living microorganisms in their own utilization of leaf litter as food. Any readily available nutrients in solution at the time of ingestion are passed into the caeca and absorbed. The bulk of the meal, though, passes into the anterior hindgut and

possibly also into the papillate region where the microbial enzymes, including the cellulases demonstrated in the present study and together with a small quantity of the isopod's own caecal enzymes, continue the breakdown of the food initiated before ingestion. Under laboratory conditions with previously starved animals this occupied up to 24 hours; under natural conditions this period may vary and be dependent on the quantity and quality of the food available. Whatever the duration, though, the typhlosole allows the isopod eventually to discharge more endogenous enzymes onto the food after a post-ingestive continuation of microbial attack.

The anterior chamber of the hindgut occupies about one half of the total length of the hindgut; it contains the bulk of ingested food and so is the major site of action by exogenous enzymes. It is significant, then, that the typhlosole delivers the endogenous enzymes at a point posterior to this site. This enables them to carry out their particular digestive functions on food already subjected to extended microbial attack, without them interfering with any further microbial action in the anterior hindgut, either on material remaining there after some has moved on into the papillate region or on newly ingested food.

The possibility that the typhlosole is concerned in some way with movement of digestive enzymes was originally put forward by Murlin (1902). He was unable to test it, with the techniques then available, and it was virtually ignored by subsequent investigators.

The distribution of phosphatases in the anterior and papillate regions of the hindgut, and the results of feeding saccharated ferrous carbonate, indicate that absorption occurs in these areas. This is to be expected, of course, since these areas are known to be sites of digestion. Phosphatases are absent from the typhlosole channels, which is understandable if these structures are used only to transport caecal enzymes. If the iron which is always present in some parts of the alimentary canal is the result of the accumulation over a long period of small amounts absorbed from the diet, which is feasible, then its differential distribution may well be instructive. Its occurrence in the caeca and papillate region could indicate that *any* soluble substances derived from the food can be, and are, absorbed in these areas. Its absence from the anterior part of the hindgut, in contrast, suggests that only products of cellulose digestion are absorbed here, making this region directly comparable to the anterior chamber of the stomach in ruminant mammals.

Previous attempts to detect cellulases in isopods, and determine their origin, involved application of biochemical, viscometric or microbiological techniques to gut extracts and yielded conflicting results. Nicholls (1931), with *Ligia oceanica,* and Newcomer (1956) with *Porcellio* sp. were unable to detect any cellulase activity, while Ray and Julian (1952) and Ray (1959a; 1959b) with *Limnoria lignorum* concluded that the cellulases they found in the caeca and elsewhere in the gut were secreted by the isopod since they found no evidence of microorganisms anywhere in the gut. Hartenstein (1964) demonstrated cellulases in caecal and whole gut extracts from *Oniscus asellus* and likewise believed them to be endogenous, although his specimens were fed on decaying sugar maple leaves. In this last study the 'caecal cellulase' could have been present in liquids pressed out of the food and passed into the caeca, although admittedly this was never found in the present work.

The cellulose-splitting activities of the microorganisms ingested by *P. muscorum* seem to be somewhat enhanced in the isopod's gut, as compared with those in control samples of uningested food. The reason for this is not immediately apparent, but it is interesting to note that similar enhancement of microbial activity has been observed in larval Bibionidae (Diptera) where two or three of the bacterial species ingested with the food proliferate in the favorable gut environment and assist digestion (Szabó, Marton and Buti, 1969). Selective survival of ingested microorganisms, and utilization of their hydrolytic capacities, occurs also in predatory leeches (Jennings and van der Lande, 1967) although here there is no particular enhancement of microbial activities.

The persistence of high cellulase activity in the freshly passed faeces of *P. muscorum* suggests that the endogenous enzymes acting in the papillate region do not kill the microorganisms. This is an interesting difference from the situation in those herbivores such as ruminant mammals which, while similarly utilizing microorganisms for cellulose digestion, and facilitating the process by delaying onset of endogenous digestive activity, do eventually digest the microorganisms and use them as a source of protein. In these instances the microorganisms are mainly symbiotic species and not, as in *P. muscorum*, free-living species ingested with the food. In most animals, of course, such micoorganisms are normally killed after ingestion. Alternatively it may be that only the microbial cellulases resist digestion in the isopod hindgut and that the microorganisms are killed and utilized as food. This would be a more efficient process and the possibility will be examined in future work.

Those features of the isopod alimentary canal which are peculiar to terrestrial species, namely the typhlosole and the subdvided hindgut, are thus believed to have a very definite functional and adaptive significance. The significance of the reduction of the midgut to the caeca, though, which is characteristic of all isopods, remains obscure. Midgut caeca are common in invertebrates, and where they play major parts in digestion and absorption the remainder of the midgut, and often the hindgut too, are reduced in size and importance (Jennings, 1972). In isopods, though, that part of the alimentary system posterior to the caeca remains fully functional and, in terrestrial species at least, has increased in importance and become the region in which the bulk of the digestive process occurs.

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Summary

1. The alimentary canal in the terrestrial isopod *Philoscia muscorum* consists of a foregut (oesophagus and proventriculus), a midgut reduced to four simple caeca, and a hindgut subdivided into anterior, papillate and sphincter regions and a rectum. The anterior region has a dorsal typhlosole whose lateral extensions form two parallel channels beneath the roof of the hindgut.

2. The isopod feeds on decaying leaf-litter. Liquids are separated in the proventriculus and passed into the caeca and absorbed; solids receive a small amount of digestive secretions containing A- and C-esterases, from the caeca, as they pass into the anterior hindgut.

3. Microorganisms already present in the food and responsible for its degradation prior to ingestion continue their action in the hindgut. They produce cellulases demonstrable by a substrate-film technique and it is believed that they are the only source of the cellulases present in the alimentary system. There is some indication that microbial activity is enhanced after ingestion by the isopod.

4. Microbial attack on the food in the anterior hindgut lasts for 20–24 hours under laboratory conditions and is followed by the major discharge of caecal enzymes. These now bypass any food remaining in the anterior hindgut, or recently ingested and passed into it, by being carried along the dorsal typhlosole channels. They are discharged into the papillate region and attack food which has already been subjected to extended microbial action.

5. Absorption occurs in both the anterior and papillate regions of the hindgut. It is suggested that only products of cellulose digestion are absorbed in the former while other digestive products are absorbed in the papillate region.

6. The typhlosole and subdivision of the hindgut are interpreted, therefore, as adaptive modifications related to a diet of decomposing leaf-litter and the utilization of the digestive capabilities of normally free-living microorganisms for the breakdown of this food, and especially of its cellulose component.

LITERATURE CITED

ALIKHAN, M. A., 1969. The physiology of the woodlouse *Porcellio laevis* Latreille (Porcellionidae, Peracarida). I. Studies on the gut epithelium cytology and its relation to maltase secretion. *Can. J. Zool.*, **47**: 65–75.

ALIKHAN, M. A., 1972. The fine structure of the midgut epithelium in the woodlouse *Porcellio laevis* Latreille (Isopoda, Porcellionidae). *Crustaceana*, Suppl. **3**: 101–109.

BAKER, J. R., 1960. *Cytological technique*, 4th ed. Methuen, London, 150 pp.

BEECHER-MOORE, B. M., 1959. The functional morphology of the alimentary canal of *Idotea emarginata* with some reference to its affinities. *Ph.D. Thesis, University of London*, London, U. K., 220 pp.

BUCHNER, P., 1965. *Endosymbiosis of animals with plant microorganisms.* Interscience, New York and London, 909 pp.

BURSTONE, M. S., 1958. Histochemical demonstration of acid phosphatase with naphthol AS-phosphates. *J. Nat. Cancer Inst.*, **21**: 523–539.

BURSTONE, M. S., AND J. E. FOLK, 1956. Histochemical demonstration of aminopeptidase. *J. Histochem. Cytochem.*, **4**: 217–226.

CHANDY, M., 1938. The histology and physiology of the intestine and hepatopancreas of two isopods, *Ligia exotica* Roux and *Armadillio elevatus* Verhoeff. *J. Royal Asiatic Soc. Bengal*, **4**: 1–15.

CLIFFORD, B., AND E. R. WITKUS, 1971. The fine structure of the hepatopancreas of the woodlouse, *Oniscus asellus*. *J. Morphol.*, **135**: 335–350.

DONADEY, M. C., 1969. La fonction absorbante des caecums digestifs de quelques Crustacés Isopodes marins, étudiée au microscope électronique. *C. R. Hebd. Seanc. Acad. Sci. Paris*, **268**(D): 1607–1609.

EDWARDS, C. A., 1974. Macroarthropods. Pages 533–554 in C. H. Dickinson and G. J. F. Pugh, Eds., *Biology of plant litter decomposition, Volume II.* Academic Press, London.

GOODRICH, A. L., 1939. The origin and fate of the entoderm elements in the embryogeny of *Porcellio laevis* Latr. and *Armadillidium nasatum* B. L. (Isopoda). *J. Morphol.*, **64**: 401–429.

HARTENSTEIN, R., 1964. Feeding, digestion, glycogen, and the environmental conditions of the digestive system in *Oniscus asellus*. *J. Insect Physiol.*, **10**: 611–621.

HASSALL, M., 1975a. Studies on the biology of *Philoscia muscorum* (Scopoli) 1763 (Crustacea: Isopoda): with particular reference to its role in a dune grassland ecosystem. *Ph.D. Thesis, University of Leeds,* Leeds, England, U. K., 300 pp.

HASSALL, M., 1975b. The functional morphology of the mouthparts and foregut in the terrestrial isopod *Philoscia muscorum* (Scopoli) 1763. *Crustaceana:* in press.

HOLDICH, D. M., 1973. The midgut/hindgut controversy in isopods. *Crustaceana,* **24**: 212–214.

HOLDICH, D. M., AND N. A. RATCLIFFE, 1970. A light and electron microscope study of the hindgut of the herbivorous isopod, *Dynamene bidentata* (Crustacea: Peracarida). *Z. Zellforsch. Mikrosk. Anat.* **111**: 209–227.

HOLT, S. J., 1958. Studies in enzyme histochemistry. *Proc. Roy. Soc. London Ser. B,* **148**: 465–532.

HRYNIEWIECKA-SZYFTER, Z., 1972. Ultrastructure of hepatopancreas of *Porcellio scaber* Latr. in relation to the function of iron and copper accumulation. *Bull. Soc. Amis Sci. Lett. Poznán, Ser. D,* **13**: 135–142.

JENNINGS, J. B., 1962. A histochemical study of digestion and digestive enzymes in the rhynchocoelan *Lineus ruber* (O. F. Müller). *Biol. Bull.,* **122**: 63–72.

JENNINGS, J. B., 1972. *Feeding, digestion and assimilation in animals,* 2nd ed. Macmillan, London, 244 pp.

JENNINGS, J. B., AND V. M. VAN DER LANDE, 1967. Histochemical and bacteriological studies on digestion in nine species of leeches (Annelida: Hirudinea). *Biol. Bull.,* **133**: 166–183.

JONES, D. A., P. C. BABBAGE AND P. E. KING, 1969. Studies on digestion and the fine structure of digestive caeca in *Eurydice pulchra* (Crustacea: Isopoda). *Mar. Biol.,* **2**: 311–320.

LASKER, R., 1959. Cellulose digestion in insects. Pages 348–355 in Dixie Lee Ray, Ed., *Marine boring and fouling organisms.* University of Washington Press, Seattle, Washington.

MURLIN, J. R., 1902. Absorption and secretion in the digestive system of the land isopods. *Proc. Acad. Natur. Sci. Philadelphia,* **54**: 284–359.

NEWCOMER, W. S., 1956. Digestive carbohydrases of the wood louse, *Porcellio. Physiol. Zool.,* **29**: 157–162.

NICHOLLS, A. G., 1931. Studies on *Ligia oceanica.* Part II. The processes of feeding, digestion and absorption, with a description of the structure of the foregut. *J. Mar. Biol. Ass. U. K.,* **17**: 675–706.

PEARSE, A. G. E., 1961. *Histochemistry: theoretical and applied,* 2nd ed. J. and A. Churchill Ltd., London, 998 pp.

RAY, D. L., 1959a. Nutritional physiology of *Limnoria.* Pages 46–61 in Dixie Lee Ray, Ed., *Marine boring and fouling organisms.* University of Washington Press, Seattle, Washington.

RAY, D. L., 1959b. Some properties of cellulase from *Limnoria.* Pages 372–396 in Dixie Lee Ray, Ed., *Marine boring and fouling organisms.* University of Washington Press, Seattle, Washington.

RAY, D. L., AND J. R. JULIAN, 1952. Occurrence of cellulase in *Limnoria. Nature,* **169**: 32.

SCHMITZ, E. H., AND T. W. SCHULTZ, 1969. Digestive anatomy of terrestrial Isopoda: *Armadillidium vulgare* and *Armadillidium nasatum. Amer. Midland Natur.,* **82**: 163–181.

SUMNER, A. T., 1968. A substrate-film method for the histochemical demonstration of cellulase. *Histochemie,* **13**: 160–168.

SUTTON, S. L., 1972. *Woodlice.* Ginn and Co. Ltd., London, 144 pp.

SZABÓ, I., M. MARTON AND I. BUTI, 1969. Intestinal microflora of the larvae of St. Mark's Fly. IV. Studies on the intestinal bacterial flora of a larval population. *Acta Microbiol. Acad. Sci. Hung.,* **16**: 381–397.

WIESER, W., 1966. Copper and the role of isopods in the degradation of organic matter. *Science,* **153**: 67–69.

WIESER, W., AND J. KLIMA, 1969. Compartmentalization of copper in the hepatopancreas of isopods. *Mikroskopie,* **22**: 1–9.

YONGE, C. M., 1926. The digestive diverticula in the lamellibranchs. *Trans. Roy. Soc. Edinburgh,* **54**: 703–718.