Diet and histophysiology of the alimentary canal of *Lumbricillus lineatus* (Oligochaeta, Enchytraeidae)

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Abstract

Lumbricillus lineatus selectively ingests masses of organic and inorganic interstitial particles from a sand-clay substratum in the upper littoral zone. Particle-masses are ingested, passed along the esophagus and into the anterior intestine where the pH becomes acid. A- and C-esterases, acid β-galactosidase, acid phosphatase and β-N-acetylglucosaminidase are present in the epithelium, while the rotating food masses are surrounded by a membrane of sulphated, acid glycoprotein. These enzymes, with the exception of acid phosphatase and the addition of aminopeptidase M, are also present in the epithelia of the mid and posterior intestinal regions where the pH is alkaline. The cells in the ventral wall of the mid intestinal region contain high concentrations of alkaline phosphatase, acid β-galactosidase and β-N-acetylglucosaminidase. The food consists of absorbed organics and bacteria with absorption and intracellular digestion occurring along the intestine, particularly in the mid ventral region.

Introduction

Information on the feeding mechanisms and digestive physiology in aquatic oligochaetes (Jeuniaux, 1969; Pandian, 1975; Michel & DeVillez, 1978) has largely been ignored as an integral part of studying their productivity and trophic interactions with other organisms of an ecosystem. Attempts to relate an oligochaete's diet with the digestive enzymes present in the gut have so far proved inconclusive (Nielsen, 1962; Kristensen, 1972; Dash *et al.*, 1981). While biochemical methods can identify enzymes accurately, the value of the information is greatly reduced and even suspect when a 'digestive' function is inferred without supporting histo- and cytochemical data. This is especially true when specimens are small and whole worms have to be homogenized. Combined biochemical and cytochemical studies have so far been restricted to *Eisenia foetida* (Van Gansen, 1962) and *Lumbricus terrestris* (Bibighaus *et al.*, 1972). Information on gut structure and feeding mechanisms has usually been reported as part of more general morphological studies (Avel, 1959; Brinkhurst & Jamieson, 1971; Jamieson, 1981). These data are important in understanding the mechanical processing of the food and in determining limiting factors such as maximum ingestible particle-size. It has long been known that not all the organic material ingested by a specimen is digested and absorbed even when selective feeding operates (Brinkhurst & Austin, 1979). Brinkhurst & Chua (1969) reported that although numerous species of bacteria were ingested, certain different species survived passage through the alimentary canal of the respective sympatric species of tubificids. Specimens of *Lumbricillus lineatus* (re-identified as *L. rivalis* by Learner, 1972) were reported to feed on detritus and microorganisms in sewage filter beds (Palka & Spaul, 1970), while Harper *et al.* (1981a, b) demonstrated conclusively that *Nais variabilis* from a similar habitat digested and assimilated bacteria. Giere (1975) re-

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ported and reviewed the information on aquatic oligochaete populations, food relations and ecological roles. He noted a direct correlation in littoral substratum distribution between *Marionina subterranea* and pennate diatoms, its food organisms. Other littoral species, *L. rivalis* (Bülow, 1957) and *L. lineatus* (Giere, 1975), were observed to be intimately associated with macrophytal debris and Giere & Hauschildt (1979) state that in their experiments the nutritive source for *L. lineatus* was algal material and not bacterial films.

In view of the variety of feeding manipulations attributed to the pharyngeal pad (Palka & Spaul, 1970) and the lack of specific information on the digestive physiology in *Lumbricillus* spp., the present study was undertaken. Its aim was to characterize the organic material ingested and demonstrate the site and sequence of certain digestive enzymes in *Lumbricillus lineatus* from an upper littoral habitat. Based upon preliminary observations on the worm's diet it was decided to concentrate on those enzymes most likely to hydrolyze the hexosamine components of glycoproteins and peptidoglycans associated with microorganisms. Inclusion of the alimentary canal description was considered necessary as it differs significantly in places from that given by Palka & Spaul (1970).

Materials and methods

Over 100 specimens of *L. lineatus* were hand-picked from sand-clay samples collected in the upper littoral zone of Lowes Cove, Maine, U.S.A. Live individuals were observed to ingest particles of the substratum and fixed at known intervals following ingestion while other specimens were fixed for histological and non-enzyme histochemical techniques. They were subsequently dehydrated in graded ethanol solutions, cleared in xylene, infiltrated in paraffin wax (m.p. 54 ° C Fisher), cut serially (6–8 μm thick sections), and stained by the appropriate protocol. Sections of specimens fixed in Hollande-Bouin fluid (Humason, 1979) were stained by Mallory's 1905 method (Lillie & Fullmer, 1976) for general histology, while acid glycoproteins and 1,2 glycol group containing compounds were demonstrated by the Alcian blue (pH 2.5 or pH 1.0) and periodic acid-Schiff reaction respectively (AB-PAS; Humason, 1979). Basic proteins were demonstrated with the mercuric bromophenol blue method (MBB; Humason, 1979), while all proteins were stained following ethanol-acetic acid fixation and Coomassie BB R250 staining (CR250; Cawood *et al.*, 1978). These histochemical techniques were also performed on aliquots of the sandy-clay substratum and observed microscopically using polarized light (Gelder, 1983).

Oligochaetes were fixed in Trump's solution (McDowell, 1978) for subsequent enzyme histochemical procedures or 4% formaldehyde buffered in 0.1 M Tris HCl, pH 7.2 when phosphatases were being sought. Fixation proceeded for 1 to 2 h at 4 ° C; the specimens were then washed in repeated changes of a 25% sucrose solution for 1 to 5 h also at 4 ° C and finally rinsed in distilled water prior to incubation. Whole specimens were incubated with naphthol AS-BI β-N-acetylglucosaminide for β-N-acetylglucosaminidase, naphthol AS-BI β-glucuronide for β-glucuronidase, L-leucyl-4-methoxy-2-naphthylamide for aminopeptidase M, naphthol AS-BI phosphate for acid and alkaline phosphatases, all of which used hexazonium-p-rosaniline for the final reaction product (see Lojda *et al.*, 1979), L-leucyl-β-naphthylamide HCl with Fast Garnet GBC for arylamidase (Burstone & Folk, 1956) and 5-bromo-4-chloro-3-indoxyl-β-galactoside for acid β-galactosidase (Lojda *et al.*, 1979). Esterases were demonstrated by Holt's indigogenic reaction using 5-bromo-3-indoxyl acetate as substrate and characterized into types A, B and C with specific inhibitors (p-chloromercuribenzoate, eserine, and β-phenylpropionic acid respectively) and activator (cysteine) (Pearse, 1972). Negative controls for all of these protocols consisted of heat inactivated specimens or the absence of enzyme substrate from the incubation media; positive controls involved simultaneous incubations of mammalian tissue sections. Following the respective incubation procedure, specimens were rinsed in distilled water, cleared in glycerine jelly (Humason, 1979) or dehydrated and cleared in xylene depending upon the solubility of the final reaction product, mounted on a slide, examined and photographed. Specimens in glycerine jelly were sectioned in a cryostat (Gelder, 1982) while those in xylene were embedded in paraffin wax and the procedure previously described was followed.

Results

Mature *L. lineatus* grow up to 0.6 mm in diameter and 7 to 10 mm in length with 38 to 42 segments (Fig. 1). In the laboratory, specimens were observed to tunnel through the interstices of sand-clay samples collected from the upper littoral zone. Providing interstitial water is present, the oligochaetes force their way through the substratum and line the resulting tunnel with a mucoid substance. The mucus binds the separated grains together and thus maintains a passage. Although the worm can pass freely back and forth in the tunnel, the walls retain their adhesiveness and will seal off the passage should opposite walls touch. The tube consists of sulphated, acid glycoprotein (AB pH 1.0) secreted from the epidermal goblet cells distributed over the surface of the worm. These are referred to as type I acid mucus secreting cells by Richards (1977). The mucoid secretion contains high levels of β-glucuronidase and low levels of acid phosphatase prior to release, while C-esterase and sometimes acid phosphatase are present both in the cytoplasm of these cells and the adjacent epidermal cells.

The prostomium and peristomium have tufts of sensory cilia distributed over their surfaces. In one or two of the epidermal cells adjacent to the cilliary tuft cells, acid β-galactosidase and A-esterase are present. Ciliary tufts and enzymes are similarly located in the epidermis of the pygidium.

Description of the alimentary canal

The alimentary canal (Fig. 1) consists of a mouth, buccal cavity, pharynx, pharyngeal glands, esophagus, intestine and subterminal anus. The gut is ciliated starting with the pharynx through to the end of the intestine; a typhlosole is absent.

The unciliated cuboidal cells which line the mouth and buccal cavity contain high concentrations of C-esterase granules. The epithelium also contains goblet cells which secrete acid glycoprotein (AB pH 2.5) into the lumen through small apertures in the thin, PAS-staining cuticle. The transition from the buccal cavity to the pharynx is marked by the cessation of the cuticle and initiation of ciliated cuboidal and columnar cells.

Three pharyngeal glands lie on either side of the esophagus, a single pair being situated in each of the posterior regions of segments 4, 5 and 6 respective-

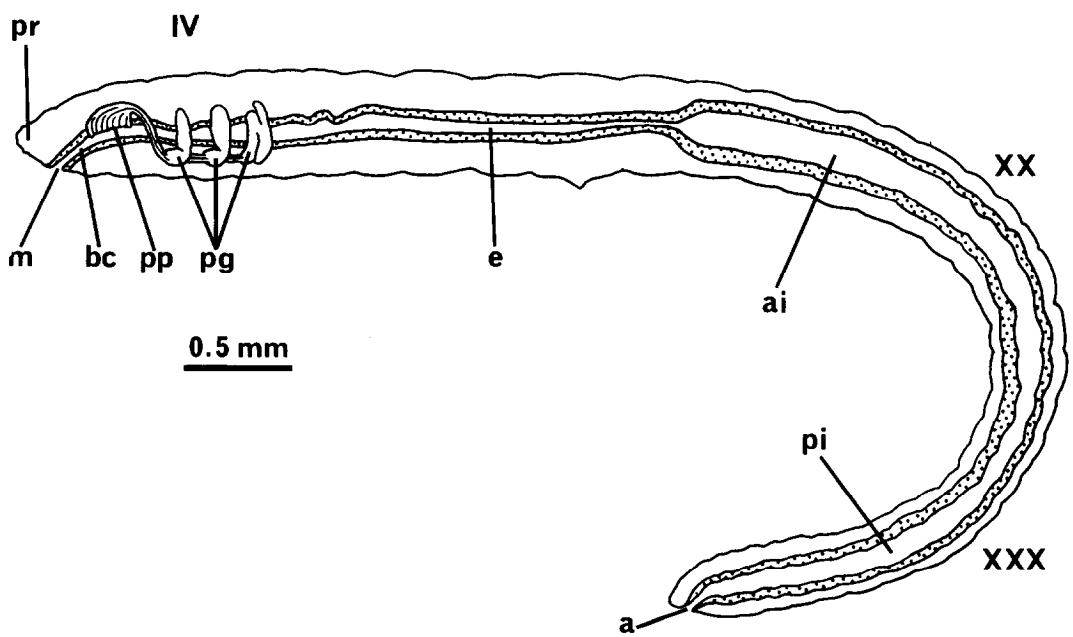
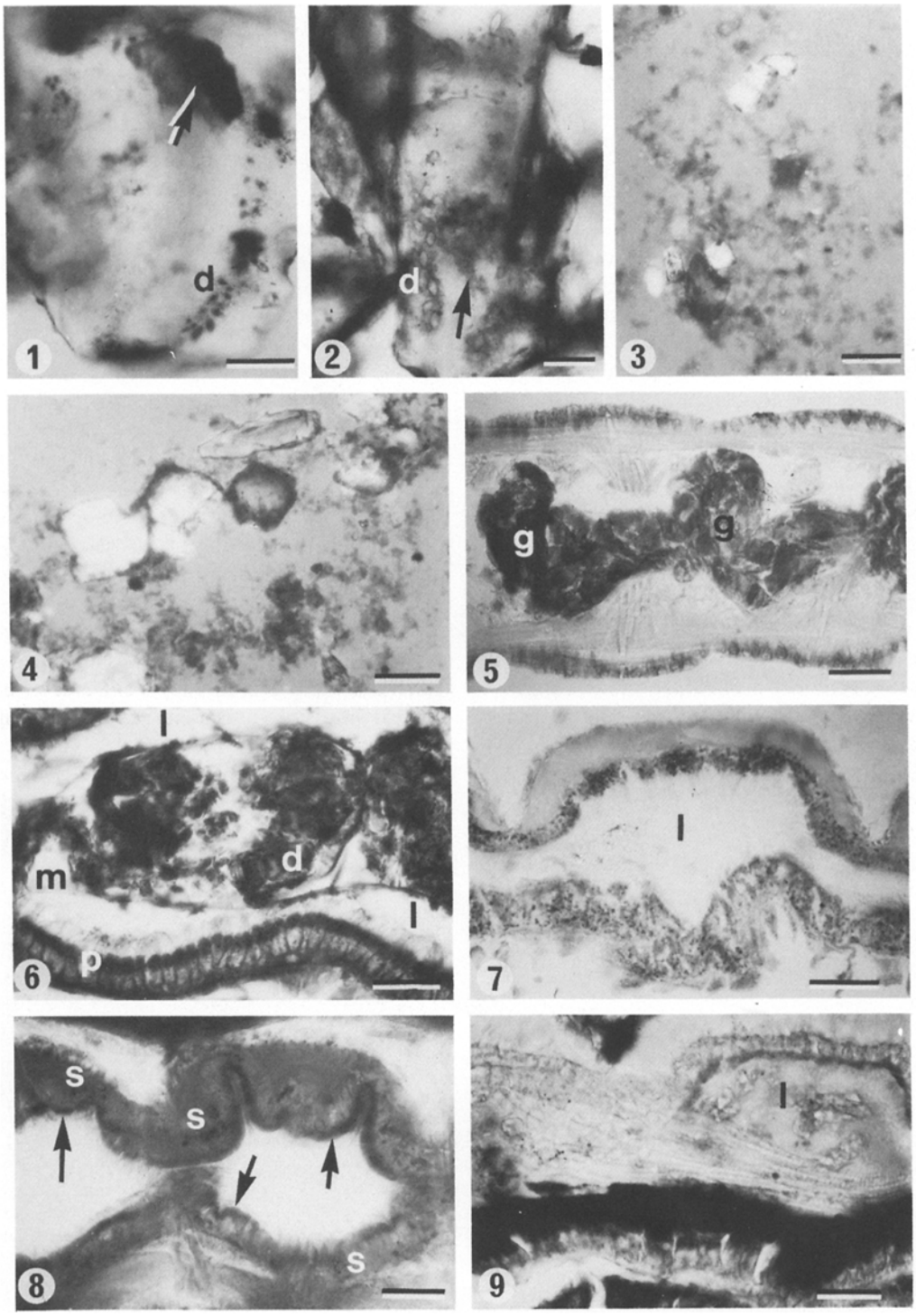


Fig. 1. A diagram of the alimentary canal and pharyngeal glands in *Lumbricillus lineatus* (a = anus, ai = anterior intestine, bc = buccal cavity, e = esophagus, m = mouth, pg = pharyngeal glands, pi = posterior intestine, pp = pharyngeal pad, pr = prostomium; IV, XX, XXX = segment numbers).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ly. A process from each gland cell passes ventrally then anteriorly along its respective side of the body, around the esophagus to converge dorsally and terminate on the surface of the pharyngeal pad (Fig. 1). The terminal region of the processes often becomes dilated from the build-up of secretion granules. The pharyngeal gland cells show a strong reaction for acid phosphatase (Plate 1.5) in addition to some scattered sites of A-esterase activity. The secretion granules are composed of an acid glycoprotein (AB pH 2.5) with a basic protein (MBB) component; they stain concomitantly with acid fuchsin and aniline blue in the Mallory technique. The secretion processes contain alkaline phosphatase and this enzyme can also be observed in the material covering the surface of the pad.

The esophagus extends from segments III to XIII and its walls consist of basophilic, ciliated cuboidal cells. A few of the epithelial cells contain low levels of acid phosphatase together with granules of C-esterase in the anterior region of the esophagus and A-esterase in the posterior region. The granules in the distal margin of these epithelial cells stain positive for basic protein (MBB) and β-N-acetylglucosaminidase. A glycocalyx of sulphated acid glycoprotein (AB pH 1.0) frequently containing alkaline phosphatase covers the lumenar surface.

Between segments XIII and XIV the narrow esophagus passes into the sligtly wider intestine; the respective lumens are usually separated by partially contracted circular muscle fibers and cilia. The epithelium of the intestine is composed predominantly of ciliated cuboidal and columnar cells with unciliated, conical 'gland cells' scattered among them. The lumenar surface of the ciliated cells is covered by a glycocalyx of sulphated acid glycoprotein (AB pH 1.0), while 2–3 μm particles of the same material are temporarily attached to the cilia (Plate 1.6). The cells of the anterior intestine in segments XIV to XIX have strongly acidophilic cytoplasm in the mid and basal regions with sulphated acid glycoprotein and acid protein (CR 250) predominating in the distal region. The acid glycoprotein granules measure 2–3 μm in diameter in the intestinal cells of the first segment (XIV); these granules become smaller in size and less numerous in the succeeding segments. The ciliated cells in most of the remaining intestine are also acidophilic but their distal regions contain either acid glycoprotein or basic protein granules. Cells in the ventral walls of segments XXVI to XXVIII have prominent vacuoles basally while acid glycoprotein and basic protein granules fill the distal region (Plate 1.6). Interspersed among these vacuolated cells are ciliated, non-vacuolar cells containing strongly staining basic protein granules. The cytoplasm of these cells appears very similar to that of the unciliated, conical gland cell. The anus is located sub-terminally on the dorsal surface of the pygidium. The anus sometimes appears sunken so that the ciliated intestinal epithelium passes into a nonciliated chamber before the lumen opens to the exterior. This is believed to be caused by the intestinal musculature's contracting during fixation.

The ciliated epithelial cells, with the exception of the ventral vacuolated cells, appeared morphologically identical even though their cytophysiology was demonstrated to vary from cell to cell. Certain ciliated cells in the dorsal wall of the anterior intestine (segments XIV to XXV) contain high concentrations of C-esterase staining granules (Plate 1.7). These and the other cells in the anterior walls also contain A-esterase staining granules with much of this isoenzyme being further characterized as acid

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Plate 1. 1. Medium sand grain with diatoms and proteinaceous fragment (arrowed), stained with Coomassie R 250 (scale 30 μm). 2. Medium sand grain showing diatoms surrounded by acid glycoprotein material (arrowed), stained by the AB pH 2.5/PAS procedure (scale 30 μm). 3 and 4. Medium and fine silt grains with associated acid glycoprotein material, stained by the AB pH 2.5/PAS procedure and illuminated by partially polarized light (scale 25 μm). 5. Longitudinal section through the pharyngeal glands, incubated for acid phosphatase (scale 20 μm). 6. Longitudinal section through a food pellet in the intestine of segment XXVI, stained by the AB pH 2.5/PAS procedure (scale 25 μm). 7. Longitudinal section through the anterior intestine in segment XVIII to show the distribution of A-esterase granules; preincubated in 1×10^{-4} M eserine, then incubated in the indoxylesterase medium and counterstained with nuclear fast red (scale 40 μm). 8. Longitudinal section through the intestine in segments XXI and XXII to demonstrate β-N-acetylglucosaminidase (arrowed) (scale 40 μm). 9. Longitudinal section through the intestine in segments XXVI and XXVII to show the distribution of alkaline phosphatase (scale 25 μm).

Abbreviations: d = diatom, g = pharyngeal gland, l = lumen of intestine, m = sulphated acid glycoprotein (? peritrophic) membrane, p = basic protein granules, s = sporozoan.

β-galactosidase (Plate 1.7). Some cells are completely filled with acid phosphatase while in others it is limited to the distal portion. β-N-acetylglucosaminidase staining granules are localized in the distal margin of the ciliated cells along the whole intestine, while high concentrations occur throughout the cytoplasm of the vacuolated cells of the ventral mid intestinal wall. From this region (segment XXIV) on, alkaline phosphatase is present in the glycocalyx, while aminopeptidase M is found scattered among β-N-acetylglucosaminidase in the distal margin. High concentrations of acid β-galactosidase staining granules are localized in some of the ventral vacuolated cells and in lower concentrations in some of the opposite cells in the dorsal wall. C-esterase granules also appear in moderate concentrations in scattered ciliated cells. Neither arylamidase nor β-glucuronidase were demonstrated in the alimentary canal.

The detailed description of the alimentary canal was complicated by the presence of spores and trophozoites of at least one species of sporozoan parasite. The trophozoites were observed in and below the epithelium of both the esophagus and intestine, while spores were localized in chlorogague tissue and gastrodermal cells. The mature trophozoites contained strongly PAS positive staining granules, suggestive of glycogen. β-N-acetylglucosaminidase and acid phosphatase activity appeared in the parasitophorous vacuoles around and sometimes inside the sporozoans located in the esophageal and anterior intestinal epithelium. High levels of these enzymes are present intracellularly in all of the sporozoans in the vacuolated cells of the mid ventral intestine but absent from those sporozoans in the posterior intestinal epithelium. The only observed host response to the parasite involved coelomocytes (granular mucocytes, after Richards, 1980) aggregating around some of the many spores in the coelom. These coelomocytes contained lysosomes in which A- and C-esterases, β-glucuronidase and acid phosphatase were demonstrated.

Diet, feeding mechanisms and digestion

The natural diet of *L. lineatus* was studied by comparing aliquots of substratum taken from around the prostomium immediately after a specimen was observed to feed with material in the alimentary canal.

The aliquots contained mineral grains of aluminosilicates (quarts, feldspar and mica) ranging from sand grain size of 350 μm in diameter to silt-clay (minimum observed diameter being 2 μm). Over half of the material in each aliquot consisted of grains, many of which were birefringent (Plate 1.1–1.4). The remaining portion of the aliquot consisted of organic material that stained PAS positive; this comprised mainly amorphous flocculant material, microorganisms and organic fragments. Blue-green algae, diatoms and small amounts of the amorphous flocculant material (Plate 1.1) stained with MBB and CR250, thus indicating their proteinaceous composition. Those microorganisms which were viable at the time of fixation stained strongly, while those in various stages of cytoplasmic dissociation only stained lightly. Diatoms and blue-green algae were invariably observed attached to the surfaces of sand and some large silt grains (Plate 1.1–1.2); they were surrounded by sulphated and non-sulphated acid glycoproteins (AB pH 1.0 and 2.5 respectively) (Plate 1.2). The interstices contained diatoms, blue-green algae, fungal hyphae, whole spores, bacteria and organic fragments together with chitinous and siliceous skeletons, and silt and clay grains (Plate 1.3–1.4). These interstitial particles were either totally or partially surrounded by a matrix of acid- and some neutral glycoproteins.

An examination of the food material in the alimentary canal showed that *Lumbricillus* ingests silt and clay grains, siliceous debris, macrofloral and chitinous fragments, diatoms, fungal hyphae, filamentous microorganisms and amorphous glycoproteins. The composition of the ingested material reflects the same variety and proportions as that observed in the less than 60 μm long fraction of the aliquot. These observations indicate that an upper particle-size limitation is imposed on the potential food, probably by the maximum dilated aperture of the mouth.

Small, unattached particles of substratum can be sucked through the mouth when the buccal and pharyngeal lumens rapidly dilate. However, the usual method of feeding involves the worm's placing its dilated mouth over a selected mass of substratum. The pharyngeal pad, its surface covered with pharyngeal gland secretions, is protracted until the food mass adheres to the pad's surface; the pad is then retracted into the pharynx and the food mass is

thus ingested. Small amounts of acid glycoproteins from the buccal epithelium become attached to the mass as it is ingested, thereby helping to protect the epithelium from abrasion and aiding in the consolidation of loose fragments. These secretions differ from those of the pad as the latter contain alkaline phosphatase. Food is detached from the pad and transported along the esophagus and into the anterior intestine by peristaltic movements and ciliary action. The particles of sulphated acid glycoprotein containing C- or A-esterases attached to the surface of the cilia do not appear to become transferred to the food. The presence of alkaline phosphatase in the glycocalyx of the esophagus with acid phosphatase, esterases and β-N-acetylglucosaminidase in the distal regions of some cells is consistent with the absorption and intracellular digestion of dissolved organics such as hexosamines.

The food-mass enters the anterior intestine and, if succeeded by further masses, is moved along the intestine. Discrete organisms in the food contain intrinsic acid phosphatase while much of the lumenar mucoids and the glycocalyx contain alkaline phosphatase. The food-masses are slowly rotated by actively beating cilia which, like those cilia in the esophagus, also have mucoid-esterase particles attached to their surfaces. After a short time the rotating food-masses are formed into spherical or cigar-shaped pellets and surrounded with a sulphated acid glycoprotein membrane (AB pH 1.0; CR 250) (Plate 1.6). During the formation of the membrane, the pH of the anterior intestinal lumen fell so that alkaline phosphatase activity ceased within the pellet and the lumen. Concomitant with the drop in pH, the concentration of localized intracellular C-esterase granules increased, along with a general rise in A-esterase, including acid β-galactosidase granules in most of the anterior intestinal cells (Plate 1.7). The distal regions of these cells also contain acid phosphatase and low levels of β-N-acetylglucosaminidase (Plate 1.8). As the level of the extracellular esterases does not increase during this period and neither acid β-galactosidase nor β-N-acetylglucosaminidase appear in the lumen, these lysosomal enzymes are deduced to be involved only in the intracellular digestion of absorbed glucosamines.

After 1 to 5 h the pH in the lumen starts to rise until it is alkaline, then alkaline phosphatase reappears in the lumenar mucoids and glycocalyx. By this time the pellets are at least in segment XXVI and adjacent to the ventral vacuolated cells. The levels of intracellular acid β-galactosidase rise and those of alkaline phosphatase greatly increase (Plate 1.9); C-esterase is also present and aminopeptidase M occurs intermittantly. Based on these observations absorption and intracellular digestion continues in the alkaline phase during the pellets' passage through the posterior intestine. The pellets are expelled through the anus where they become attached to the adhesive wall of the worm's mucoid tube. Most of the microphyta and fungi that escaped mechanical damage prior to ingestion appear to be still viable upon egestion from the worm. No examination of bacterial flora was performed on the pellets.

Discussion

The ultrastructure of the pharyngeal glands in the enchytraeids, *Enchytraeus albidus* (Reger, 1967) and *Lumbricillus lineatus* (Ude, 1971, 1977), showed that secretion bodies were transported to the pharyngeal pad from the cell bodies inside their respective cytoplasmic processes. A similar arrangement was found in the present study. Acid phosphatase and A-esterase activity in the pharyngeal gland cells of *L. lineatus* and inosindiphosphate, acid phosphatase, thiaminpyrophosphatase and peroxidase in *L. lineatus* (Ude, 1975) appear to be concerned with secretion synthesis rather than being secreted as digestive enzymes. Reger (1967) and Ude (1971, 1977) both suggest secretion granules are moved along the cellular processes by cytoplasmic flow and this is consistent with the strong reaction obtained for alkaline phosphatase in *L. lineatus*. Although the pharyngeal gland secretion is an acid glycoprotein similar to that in *Eisenia foetida* (Van Gansen, 1962) no amylase was noted; proteases (Keilin, 1920) and trypsin (Bibighaus *et al.*, 1972) demonstrated in *Lumbricus terrestris* were similarly absent.

The primary function of the pharyngeal pad secretion appears to be as an adhesive that is strong enough to hold food particles against the pad while the protruded pharynx is retracted. The pad was never observed to act as a 'sucker' (Palka & Spaul, 1970). Giere & Hauschildt (1979) noted a 'bruising' action, however this was intended to read 'brows-

ing' (Giere, pers. commun.). The possibility of ingested grains being abraded together as a mechanism for dislodging epi-organisms is prevented by the mucoid material which completely or partially surrounds their surfaces. *Lumbricillus lineatus* is a selective feeder (Giere & Hauschildt, 1979); it ingests only mineral grains and detrital masses less than 60 μm in diameter.

Determination of food particle sizes, characterization of the organic material ingested and preferences are among the essential parameters required to understand the nutritional role of benthic deposit-feeders (Levinton, 1980). Whitlatch (1981) characterized the substratum on the basis of inorganic and organic particle morphology and found that most of the organic material contained 1,2 glycol groups (PAS positive). The organic material in the substratum samples in this study proved similar to those previously reported and demonstrated by histochemical techniques by other workers (Frankel & Mead, 1973; Whitlatch & Johnson, 1974), predominantly acid glycoproteins and occasionally proteins adsorbed onto mineral grains. The abundance of acid glycoprotein led Hobbie & Lee (1980) to suggest that such extracellular particulate matter may make up most of the food of detritivores. Any deficiency in nitrogen could be supplemented by the adsorbed proteins and microflora.

β-glucuronidase, β-N-acetylglucosaminidase and acid β-galactosidase were chosen as indicator enzymes as they are known to have key roles in the anabolism and catabolism of glycoproteins (Kornfeld & Kornfeld, 1980; Rodén, 1980; Berger *et al.*, 1982). The most likely effect of β-N-acetylglucosaminidase and β-N-acetylgalactosaminidase, which are indistinguishable by the histochemical technique used (Lojda *et al.*, 1979), is the liberating of the proteoglycan monomers from the hyaluronic acid backbone (Rodén, 1980). Under certain conditions the exposed hyaluronic acid chain can then be hydrolyzed by exo-β-glucuronidase and exo-β-N-acetylglucosaminidase to monosaccharide units (Rodén, 1980). These processes would provide a significant, if variable degree of degradation of most glycoproteins including those surrounding bacteria. Consequently endo-β-N-acetylglucosaminidases, one of which is referred to as lysozyme, could hydrolyze specific sites on the peptidoglycan chain which forms the chief component of the bacterial cell wall (Kimball, 1983). Such a breach in the cell wall would remove the chance of pathogenicity following uptake and concomitantly expose the cytoplasm of the bacteria as a food source.

The intracellular enzymes demonstrated in the glycocalyx and distal region of the esophageal epithelial cells suggest absorption of nutrients for intracellular digestion. However, as food masses usually took 30 to 60 s to pass from the mouth to the anterior intestine, only dissolved, small molecules such as hexosamines would be available for uptake so soon after ingestion.

The constant rotation of the food masses by the epithelial cilia results in the masses' becoming ovoid or elongate and surrounded by a heterogeneous membrane of Alcian blue pH 2.5 and PAS staining material. A comparable membrane in *Enchytraeus albidus*, referred to as the peritrophic membrane, lacks chitin (Peters, 1968) whereas in *Dero obtusa* (Peters, 1968) and *Lumbricus terrestris* the membrane contains chitin. Aminopeptidase was demonstrated in the peritrophic membrane of *Lumbricus* (Vierhaus, 1971) and thought to have a role in digestion.

It is difficult to believe that extracellular digestion does not occur; however no secretion of extracellular or intrinsic enzymes was demonstrated. This suggests that enzymes probably were acting upon the food but were different from the ones sought. It is surprising that β-glucuronidase was entirely absent from the digestive enzyme complement of *L. lineatus*. Haase (1969) reported β-glucuronidase from the typhlosole of lumbricids. A typhlosole is absent in these specimens of *L. lineatus* (reported as *L. rivalis*; Palka & Spaul, 1970), but it is not thought that these two facts are connected. β-glucuronidase was reported in the nematode *Monhystera denticulata* (Jennings & Deutsch, 1975) and the polychaete *Histriobdella homari* (Jennings & Gelder, 1976), and its presence interpreted as being consistent with a bacterial diet. The post-coupling method (Pearse, 1972) used in the cited works was compared with the simultaneous coupling technique which uses naphthol AS-BI β-glucuronide with hexazonium pararosaniline (Lojda *et al.*, 1979) on specimens of *H. homari*. The post-coupling method demonstrates sites of β-glucuronidase in the stomach epithelium (Jennings & Gelder, 1976), while the simultaneous coupling technique visualizes the enzyme in the acid glycoprotein secreting (6th) pair of the salivary glands;

positive controls for this study consisted of mouse kidney sections (Gelder, unpubl. obs.). These observations could suggest that two of the several isoenzymes of β-glucuronidase referred to by Lojda *et al.* (1979) were being demonstrated by the respective substrates. This appears highly unlikely as the same authority reports that 6-bromo-2-naphthol will give positive reactions in enzyme inactivated sections and false-negatives in cells containing β-glucuronidase; the validity of results obtained using this substrate must be questioned.

Sulphate acid glycoprotein and β-glucuronidase occur together in the 6th pair of salivary glands of *H. homari* and in the type I acid mucus secreting cells in the epidermis of *L. lineatus* (nomenclature after Richards 1977). This latter cell-type can be demonstrated in living specimens using neutral red stain (see Kossmagk-Stephan, 1984). It would appear that the β-glucuronidase keeps the mucoid components unpolymerized so that upon secretion, whether into the buccal cavity or onto the tunnel's wall, the enzyme is inactivated and the glycoprotein units polymerize. The converse was reported by Corner *et al.* (1960) where β-glucuronidase was related to the digestion of algal sulphated polysaccharides. The absence of this enzyme from the alimentary canal of *L. lineatus* indicates that hydrolysis of β-glucuronidase units are not present or are not as important as in the study just cited. However, as Hobbie & Lee (1980) note, it is the release of the nitrogen containing sugar units, namely N-acetylglucosamine and N-acetylgalactosamine, that are nutritionally important.

The sites of β-N-acetylglucosaminidase, which according to Lojda *et al.* (1979) probably include β-N-acetylgalactosaminidase, occur along the lumenar margin of the intestinal epithelium and in high concentrations throughout the vacuolated cells in the ventral wall of the mid intestine. Although no microorganisms were seen to be phagocytosed into the mid-ventral intestinal cells, bacteria-size particles could have been taken up unobserved and the high level of β-N-acetylglucosaminidase activity could reflect their intracellular digestion as hypothesized earlier. Such active uptake would certainly be supported by the intense alkaline phosphatase activity demonstrated along the lumenar margin. Acid β-galactosidase was detected in the intestinal epithelium and was also found in a similar location in *Mesenchytraeus glandulosus* (Nielsen, 1962). β-galactosidase is known to act upon yeast and bacteria (Gottschalk, 1958) and particularly upon galactolipids found in algae (Benson & Shibuya, 1962). The histochemical technique used for A-esterase detection in the present study also demonstrates acid β-galactosidase activity. A comparable visualization of A-esterase, interpreted as 'endopeptidase', was observed on the surface of yeast cells, detrital and other microorganismal food particles in the stomach of *Dinophilus gyrociliatus* (Jennings & Gelder, 1969). In view of the results in the present study and the nature of the food substrate in the stomach of *D. gyrociliatus* it would appear that the A-esterase demonstrated also contained acid β-galactosidase. Evidence of proteases in *L. lineatus* is restricted to very low levels of aminopeptidase M and possibly endopeptidase activity intracellularly. The combination of β-N-acetylglucosaminidase, acid β-galactosidase and A- and C-esterases indicates the importance of intracellular digestion in *L. lineatus* but does not rule out an extracellular phase.

In view of the intricacies demonstrated and those suggested in resource partitioning of microorganisms by hydrobids (Hylleberg, 1975; Lopez & Levinton, 1978) and the review of particle feeding by deposit feeders (Levinton, 1980), further studies are needed on the nutrition of *L. lineatus*. These studies will also have to contend with the possibility of different nutritional regimes in the identifiable genetic populations of *L. lineatus* in the various intertidal levels (Christensen, 1980; see Christensen, 1984). Therefore, in determining the full trophic impact of *L. lineatus* on the intertidal ecosystem, it is important that any trophic variations within the intertidal populations are not lost and data become apparently contradictory.

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References

Avel, M., 1959. Classe des annélides oligochaetes. In P.-P. Grassé (ed.), Traité de Zoologie 5. Masson, Paris: 224–470.

Benson, A. A. & I. Shibuya, 1962. Surfactants lipids. In R. A. Lewis (ed.), Physiology and biochemistry of algae. Academic Press, N.Y.: 371–383.

Berger, E. G., E. Buddecke, J. P. Kamerling, A. Kobata, J. C. Paulson & J. F. G. Vliegenthart, 1982. Structure, biosynthesis and functions of glycoprotein glycans. Experientia 38: 1129–1162.

Bibighaus, C. L., E. J. DeVillez & A. L. Allenspach, 1972. Histochemical localization of production and secretion of trypsin in the earthworm Lumbricus terrestris. Z. Zellforsch. 130: 429–439.

Brinkhurst, R. O. & M. J. Austin, 1979. Assimilation by aquatic Oligochaeta. Int. Revue ges. Hydrobiol. 64: 245–250.

Brinkhurst, R. O. & K. E. Chua, 1969. Preliminary investigation of the exploitation of some potential nutritional resources by three sympatric tubificid oligochaetes. J. Fish. Res. Bd. Can. 26: 2659–2667.

Brinkhurst, R. O. & B. G. M. Jamieson, 1971. Aquatic Oligochaeta of the world. Oliver & Boyd, Edinb., 860 pp.

Bülow, T., 1957. Systematisch-autökologische Studien an eulitoralen Oligochaeten der Kimbrischen Halbinsel. Kieler Meeresforsch. 13: 69–116.

Burstone, M. S. & J. E. Folk, 1956. Histochemical demonstration of aminopeptidase. J. Histochem. Cytochem. 4: 217–226.

Cawood, A. H., U. Potter & H. G. Dickinson, 1978. An evaluation of Coomassie brilliant blue as a stain for quantitative microdensitometry of protein sections. J. Histochem. Cytochem. 26: 645–650.

Christensen, B., 1980. Constant differential distribution of genetic variants in polyploid parthenogenetic forms of Lumbricillus lineatus (Enchytraeidae, Oligochaeta). Hereditas 92: 193–198.

Christensen, B., 1984. Asexual propagation and reproductive strategies in aquatic Oligochaeta. In G. Bonomi & C. Erséus (eds.), Aquatic Oligochaeta. Dev. Hydrobiol. (this volume).

Corner, E. D. S., Y. A. Leon & R. D. Bulbrook, 1960. Sulphatases and β-glucuronidase in marine invertebrates. J. mar. biol. Ass. U.K. 39: 51.

Dash, M. C., B. Nanda & P. C. Mishra, 1981. Digestive enzymes in three species of Enchytraeidae (Oligochaeta). Oikos 36: 316–318.

Frankel, L. & D. J. Mead, 1973. Mucilaginous matrix of some estuarine sands in Connecticut. J. sedim. Petrol. 43: 1090–1095.

Gelder, S. R., 1982. Sectioning small invertebrates containing enzyme histochemical azo-dye final reaction products. Proc. r. microsc. Soc. 17: 93–94.

Gelder, S. R., 1983. Enhancement of histochemically demonstrated organic materials on sand-silt grains using polarized light. Tech. Inf. Bull., Leitz, USA 1: 11–12.

Giere, O., 1975. Population structure, food relations and ecological role of marine oligochaetes, with special reference to meiobenthic species. Mar. Biol. 31: 139–156.

Giere, O. & D. Hauschildt, 1979. Experimental studies on the life cycle and production of the littoral oligochaete Lumbricillus lineatus and its response to oil pollution. In E. Naylor & R. G. Hartnoll (eds.), Cyclic phenomena in marine plants and animals. Pergamon Press, Oxford: 113–122.

Gottschalk, A., 1958. The enzymes controlling hydrolytic, phosphorolytic and transfer reactions of the oligosaccharides. Handb. Pfl. Physiol. 6: 87–124.

Haase, E., 1969. Zur Histophysiologie des Regenwurmdarmes. Zool. Anz., Suppl. 33: 535–539.

Harper, R. M., J. C. Fry & M. A. Learner, 1981a. Digestion of bacteria by Nais variabilis (Oligochaeta) as established by autoradiography. Oikos 36: 211–218.

Harper, R. M., J. C. Fry & M. A. Learner, 1981b. A bacteriological investigation to elucidate the feeding biology of Nais variabilis (Oligochaeta: Naididae). Freshwat. Biol. 11: 227–236.

Hobbie, J. E. & C. Lee, 1980. Microbial production of extracellular material: importance in benthic ecology. In K. R. Tenore & B. C. Coull (eds.), Marine benthic dynamics. Belle W. Baruch Library, Univ. S. Carolina: 341–346.

Humason, G. L., 1979. Animal tissue techniques. 4th ed. Freeman & Co., San Francisco, 661 pp.

Hylleberg, J., 1975. Resource partitioning on basis of hydrolytic enzymes in deposit-feeding mud snails (Hydrobiidae). 2. Studies on niche overlap. Oecologia 29: 115–125.

Jamieson, B. G. M., 1981. The ultrastructure of the Oligochaeta. Academic Press, N.Y., 462 pp.

Jennings, J. B. & A. Deutsch, 1975. Occurrence and possible adaptive significance of β-glucuronidase and arylamidase ('leucine aminopeptidase') activity in two species of marine nematodes. Comp. Biochem. Physiol. 52A: 611–614.

Jennings, J. B. & S. R. Gelder, 1969. Feeding and digestion in Dinophilus gyrociliatus (Annelida: Archiannelida). J. Zool., Lond. 158: 441–451.

Jennings, J. B. & S. R. Gelder, 1976. Observations on the feeding mechanism, diet and digestive physiology of Histriobdella homari van Beneden, 1858: an aberrant polychaete symbiotic with North American and European lobsters. Biol. Bull. mar. biol. Lab., Woods Hole 151: 489–517.

Jeuniaux, C., 1969. Nutrition and digestion. In M. Florkin & B. T. Scheer (eds.), Chemical zoology 4. Academic Press, N.Y.: 69–91.

Keilin, D., 1920. On the pharyngeal or salivary gland of the earthworm. Q. J. microsc. Sci. 65: 33–61.

Kimball, J. W., 1983. Biology. 5th ed. Addison-Wesley Publishers, Reading, Mass., 747 pp.

Kornfeld, R. & S. Kornfeld, 1980. Structure of glycoproteins and their oligosaccharide units. In W. J. Lennarz (ed.), The biochemistry of glycoproteins and proteoglycans. Plenum Press, N.Y.: 1–33.

Kossmagk-Stephan, K.-J., 1984. A method of identifying immature specimens of marine Enchytraeidae (Oligochaeta) by vital staining of epidermal glands. In G. Bonomi & C. Erséus (eds.), Aquatic Oligochaeta. Dev. Hydrobiol. (this volume).

Kristensen, J. H., 1972. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar. Biol. 14: 130–142.

Learner, M. A., 1972. Laboratory studies on the life-histories of four enchytraeid worms (Oligochaeta) which inhabit sewage percolating filters. Ann. appl. Biol. 70: 251-266.

Levinton, J. S., 1980. Particle feeding by deposit-feeders: models, data and a prospectus. In K. R. Tenore & B. C. Coull (eds.), Marine benthic dynamics. Belle W. Baruch Library, Univ. S. Carolina: 423-440.

Lillie, R. D. & H. M. Fullmer, 1976. Histopathologic technic and practical histochemistry. 4th ed. McGraw-Hill, N.Y., 942 pp.

Lojda, Z., R. Gossran & T. H. Schiebler, 1979. Enzyme histochemistry. Springer-Verlag, N.Y. 339 pp.

Lopez, G. R. & J. S. Levinton, 1978. The availability of microorganisms attached to sediment particles as food for Hydrobia ventrosa Montagu (Gastropoda: Prosobranchia). Oecologia 32: 263-275.

McDowell, E. M., 1978. Fixation. In A. Trump & J. Jones (eds.), Diagnostic electron microscopy. J. Wiley & Sons, N.Y.: 113-139.

Michel, C. & E. J. DeVillez, 1978. Digestion. In P. J. Mill (ed.), Physiology of annelids. Academic Press, N.Y.: 509-554.

Nielsen, C. O., 1962. Carbohydrases in soil and litter invertebrates. Oikos 13: 200-215.

Palka, J. & E. A. Spaul, 1970. Studies of feeding and digestion in the enchytraeid worm Lumbricillus lineatus Müll. in relation to its activity in sewage bacteria beds. Proc. Leeds phil. lit. Soc. 10: 45-59.

Pandian, T. J., 1975. Mechanisms of heterotrophy. In O. Kinne (ed.), Marine ecology, 2, 1. J. Wiley & Sons, N.Y.: 61-249.

Pearse, A. G. E., 1972. Histochemistry: theoretical and applied, 2. 3rd ed. Churchill Livingstone, Edinb., 761-1518.

Peters, W., 1968. Verkommen, Zusammensetzung und Feinstruktur peritrophischer Membranen im Tierreich. Z. Morph. Tiere 62: 9-57.

Reger, J. F., 1967. A fine structure study on the organization and innervation of pharyngeal glands and associated ciliated epithelium in the annelid Enchytraeus albidus. J. Ultrastruct. Res. 20: 451-461.

Richards, K. S., 1977. Structure and function in the oligochaete epidermis (Annelida). Symp. zool. Soc. Lond. 39: 171-193.

Richards, K. S., 1980. The histochemical and ultrastructure of the coelomocytes of species of Lumbricillus, and observations on certain other enchytraeid genera (Oligochaeta: Annelida). J. Zool., Lond. 191: 557-577.

Ude, J., 1971. Untersuchungen zur Ultrastruktur des Septaldrüsensystems von Pachydrilus lineatus (Annelida-Oligochaeta). Acta histochem., Suppl. 11: 217-226.

Ude, J., 1975. Elektronenmikroskopisch-enzymhistochemische Untersuchungen zum Sekretionsprozess in den Septaldrüsenzellen von Pachydrilus lineatus. Acta histochem., Suppl. 15: 177-186.

Ude, J., 1977. Licht- und elektronenmikroskopische Untersuchung des Septaldrüsensystems von Pachydrilus lineatus (Annelida-Oligochaeta). Zool. Jb. Allgem. Zool. Physiol. Tiere 81: 42-82.

Van Gansen, P., 1962. Structures et fonctions du tube digestif du lombricien Eisenia foetida Savigny. Ann. Soc. r. zool. Belg. 93: 1-121.

Vierhaus, H., 1971. Über peritrophische Membranen und andere chitinhaltige Strukturen bei Anneliden, unter besonderer Berücksichtigung von Lumbricus terrestris L. Doct. Diss.

Whitlatch, R. B., 1981. Animal-sediment relationships in intertidal marine benthic habitats: Some determinants of deposit-feeding species diversity. J. exp. mar. Biol. Ecol. 53: 31-45.

Whitlatch, R. B. & R. G. Johnson, 1974. Methods for staining organic matter in marine sediments. J. sedim. Petrol. 44: 1310-1312.