

# GEOMETRY OF NUTRIENT ABSORPTION IN DEPOSIT FEEDERS.

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## Abstract

Gut luminal surface area and nutrient uptake were observed in six species of deposit feeders: two holothuroids, *Parastichopus californicus* and *Molpadia intermedia*; one irregular echinoid, *Brisaster latifrons*; and three polychaetes, *Arenicola marina*, *Amphitrite johnstoni* and *Thelepus crispus*. Epithelial folding was quantified by estimating the surface roughness relative to a smooth cylinder. Variation in density of nutrient transporter sites were estimated as the degree to which radio-labelled D-glucose and amino acids (AA) accumulated in gut tissue against steep gradients.

The anterior caeca of *Arenicola* and *Brisaster*, and foregut of *Parastichopus* were highly folded with low to moderate absorptive potential inferring an enzyme-secretory function for these gut sections. Midgut and posterior sections of *Amphitrite* and *Molpadia* had slightly higher epithelial surface area sometimes with accompanying high absorptive potential. Enhanced anterior surface area in some species suggests that enzyme delivery to sediment needs to occur rapidly to quickly hydrolyze a very dilute substrate. However, regional specialization was not the norm as all gut sections display absorptive capacity.

Nitrogen sources were amassed by gut tissue in spite of steep cell-to-lumen concentration gradients. D-glucose, the only carbon source tested, was not accumulated in gut tissue. All species accumulated L-tyrosine against a 50  $\mu$ M concentration gradient. All but *Brisaster* stockpiled L-methionine against a 500  $\mu$ M gradient. We found unusually large accumulation of methionine and

tyrosine by the terebellid polychaetes which, due to smaller gut diameters, have only half the specific (per gut length) luminal surface area of larger species. To achieve this *Amphitrite* and *Thelepus* probably maintain greater methionine and tyrosine transporter densities than other species. We link the propensity for methionine uptake by these intertidal tube-dwellers to an osmoregulatory function. Specifically, the conversion of methionine to the well known osmolyte taurine. Fluctuating osmolality of tube water from winter rain dilution or summer evaporative concentration of salinity and metabolites during low tide, followed, on re-immersion, by acclimation to ambient tidal water, continuously changes the direction and magnitude of osmotic gradients. Organic osmolytes such as taurine mitigate the effects of high solute concentrations within the gut by protecting the conformational structure of digestive enzymes.

*Key words:* Deposit feeder; Gut; Luminal surface area; Nutrient absorption; Holothuroid; Annelid;

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## 1. Introduction

Marine deposit feeders obtain food by ingesting sediment, other deposited material, and associated microbes. Although sources of nutrition to few deposit feeders are known, it is well established that the material they ingest is nutritionally dilute. It consists primarily of mineral grains, and much of the small percentage of organic matter in sediments is refractory (Jumars et al., 1984; Lopez & Levinton, 1987; Mayer, 1989). Despite the low quality of their food, deposit feeders are spectacularly successful at extracting nutrients from sediments. They are abundant in coastal and shelf sediments, and dominate bathyal and abyssal depths (Jumars et al., 1989).

The question of how deposit feeders satisfy their nutritional needs despite such low food quality has intrigued biologists for decades. Research on the morphologies (Foster & Hodgson, 1996) and enzyme activities of digestive tracts (e.g., Feral & Massin, 1982) has contributed significantly to understanding the adaptations of deposit feeders to their unusual diet. A theory of digestion was developed in a series of papers that synthesized information on gross digestive-tract

morphology, chemical-reactor design, and feeding rates (Penry & Jumars, 1986, 1987; Penry, 1989; Penry & Jumars, 1990; Dade et al., 1990). Results of these studies suggest that the operation of most portions of most deposit-feeders digestive tracts are best modeled as simple, tubular chemical reactors termed continuous, plug-flow reactor (PFRs; Penry & Jumars, 1986, 1987). Material in an ideal PFR does not mix significantly along the flow axis but is perfectly mixed radially and flows at a constant rate from anterior to posterior. Concentrations of food decrease continuously from anterior to posterior. Hydrolysis rates are high at the anterior end and decline posteriorly along with reactant concentrations.

Two other ideal (in the sense of simply described) chemical-reactor designs are used to model digestive tracts: the batch reactor and the continuous-flow, stirred-tank reactor (CSTR). In a CSTR, material is continually taken in, mixed, and removed. Concentrations of food and enzymes are diluted immediately to a constant level upon inflow and are at the same level at the outflow end. Reaction rate remains constant throughout an ideal CSTR. In a batch reactor, the reactants, i.e., food and enzymes, are added to a container and mixed continuously. The reaction is allowed to proceed for a set period, after which products and unreacted material are removed. The concentration of reactants changes over time, and the operation of a batch reactor is interrupted periodically to empty and refill the container. The governing equations are very similar to those for plug flow and are best applied to animals eating food in discrete meals that are not mixed with each other as digestion proceeds (Penry & Jumars, 1987).

The PFR and CSTR permit continuous feeding, requiring no extra time for emptying and refilling the container. It is hypothesized that a PFR is generally the most appropriate model for deposit feeders because, in addition to permitting continuous feeding, it requires a shorter throughput time than the CSTR for an equal conversion of reactants to products (Penry & Jumars, 1986, 1987; Martnez del Rio et al., 1994). More recently, Jumars (in manuscript) in an extension of this modeling work has demonstrated that this advantage is amplified by the absorptive step that follows hydrolysis. A PFR can achieve 20% greater absorption and can do so in 56% of the time relative to a CSTR. Mixing in a CSTR dilutes reactants to a lower, constant concentration

immediately at the anterior end and creates a greater probability that hydrolysis products will be egested before they can be absorbed. Although in a PFR reactant concentrations, and therefore reaction rates, decline from anterior to posterior, on average they exceed the concentrations in a CSTR. A CSTR would require a greater throughput time, or a greater reactor volume given a constant throughput rate, than a PFR to match its production-rate capacity. Jumars (in manuscript) has also found that much of the advantage of the PFR disappears if the gut-averaged rates of hydrolysis and absorption are poorly matched. This finding leads naturally to the question of how such balance might be achieved in an animal feeding on nutritionally dilute material.

To date provisional model assignments and other inferences have been made primarily on the basis of the gross external morphologies of digestive tracts. Internal gut morphologies, however, provide other valuable clues of gut function. In particular, it is important to examine the morphology of the digestive epithelium, the gut tissue most directly in contact with ingested material. Although the outer gut wall is relatively smooth, the configuration of the digestive epithelium varies among gut regions and among guts of different species. Epithelial folding can have important functional consequences by affecting such parameters as the volume of the gut lumen occupied by sediment and fluid and the number of secretory or absorptive cells per unit of gut length. The purpose of the present paper is to investigate the structure and function of the digestive epithelium in deposit feeders and the applicability of chemical reactor models. Unlike the majority of prior studies, we deliberately crossed taxonomic boundaries with simple and constant methodology in order to seek generalizations for deposit feeders. Epithelial morphology was examined using general histology, and the degree of epithelial folding was quantified by estimating the surface area of epithelium. Qualitative assessment of secretory and absorptive cell-types and quantitative measurement of luminal nutrient uptake guided our understanding of structure-function linkage. The study animals included two holothuroids, *Parastichopus californicus* and *Molpadia intermedia*; one irregular echinoid, *Brisaster latifrons*; and three polychaetes, *Arenicola marina*, *Amphitrite johnstoni* and *Thelepus crispus*. We combined our

histological and absorption information with published data on gross morphology and biochemical activities (e.g. Feral, 1989) to draw inferences for future testing.

## 2. Materials and methods

### 2.1. Species description

A surface deposit feeder from the Darmiscotta estuary of Maine, *Amphitrite johnstonia* (terebellida:polychaeta) is also found in the British Isles (Dales, 1955). Animals were collected from mudflats. Individuals occupy tubes made of cemented mud or tough cartiligenous material. Unrelaxed individuals for the absorption measurements ranged from 10-18 cm long x 1cm wide with guts from 20-36 cm long.

*Arenicola marina* (arenicolida:polychaeta), the common lugworm, was collected from South Port Island and Lubec, Maine. Individuals for the absorption measurements ranged in size from 9-15 cm long x 1 cm diameter with an average gut length of 13 cm. Aspects of its biology have been described in many papers including: feeding (Jacobsen, 1967), gut anatomy (Kermack, 1955), digestion (Longbottom, 1970), nutrient absorption (Bamford & Stewart, 1973a,b) and population dynamics (Flach & Beukema, 1994).

The tentaculate surface deposit-feeder *Thelepus crispus* (terebellida:polychaeta) were collected from the +2 to +4 ft tide level of a pebble-mud habitat San Juan Island, Washington (Woodin, 1974). Tubes containing individuals were scraped from the underside of larger, partially submerged, rocks. Unrelaxed individuals for the absorption measurements ranged from 5 - 16cm long with thoracic sections to 1cm wide. Gut length paralleled body length with four anatomically distinct sections (Penry & Jumars, 1990).

The motile, surface-deposit feeding sea cucumber *Parastichopus californicus* (aspidochirotida : holothuroidea) are commonly found subtidally along inshore rocky shorelines of northern Puget Sound and British Columbia, Canada (Cameron & Fankboner, 1989). Our specimens were collected near the Friday Harbor Laboratories, San Juan Island, Washington, USA. Nominal

animal size was 30cm long x 10cm diameter with guts to 90cm length. This species supports a local fishery.

*Brisaster latifrons* is a geographically wide-ranging irregular or “heart” urchin (spatangoid:echinoidea) commonly found in mud environments at water depths ranging from 60 - 1800 meters along the Pacific coast of North America (Lie & Kisker, 1970; McCauley & Carey, 1967), Asia (Kanazawa, 1991) and the Bering Sea (Clark, 1917). Individuals (shell size 6cm x 6cm x 3cm height; average gut length 34 cm) collected for our measurements were found in the soupy, surface sediment layer, in water depths of 200 meters of central Puget Sound, Washington, USA (Nichols, 1975). Not only of interest to biologist, *Brisaster*'s feeding and movement effects on current (Nichols et al., 1989) and ancient (Howard et al., 1974) continental shelf sediments has also attracted attention from sediment transport geologist and paleontologists (Kanazawa, 1992). The description of gut morphology of sand-dwelling spatangoids by Buchanan et al. (1980) also applies to *Brisaster*. Plante & Jumars (1992) describe *Brisaster*'s gut as a micro-environment for microbial growth.

The apodus mud-dwelling sea cucumber *Molpadia intermedia* (molpadiida:holothuroidea) was often collected in the same grab samples with *Brisaster*. Individuals used in the absorption measurements were 14 cm by 3 cm diameter and possessed guts up to 30 cm long. Nichols (1975) studied this subsurface deposit feeder's population dynamics and Plante & Jumars (1992) its gut as a suitable habitat for microbes. A congener *Molpadia oolitica* is an important source of sediment reworking in Cape Cod Bay, Massachusetts (Rhoads & Young, 1971).

## 2.2. Luminal surface area

After specimens were relaxed in MgCl<sub>2</sub>, gut segments were excised and embedded in Paraplast embedding medium (m.p. 54 °C). Histological sections, 7.5 micrometers thick, were cut on a microtome, mounted on slides, and stained with Gomori's trichrome stain. A video camera attached to a microscope and image analyzer was used to estimate surface area enhancement due to epithelial folding in cross and longitudinal sections. We did not investigate whether folding showed

self-similarity and thus could be described fractally, nor did we more generally examine degree of folding as a function of scale, but our data provide single beginning points in each species and gut section for such study. After dividing digestive tracts into segments 0.5 - 1.0 cm long, we calculated epithelial surface area of each gut segment as the product of the average perimeter of the epithelium in cross section and the average length of epithelium in the gut segment. To obtain average perimeters and lengths of epithelium, at least five cross sections and five longitudinal sections were measured in each gut region. Although epithelial folds were measured in calculating epithelial surface areas, microvillae were not, as they could not be clearly resolved in histological sections. The surface area of epithelium is therefore underestimated by the measurements presented here. Relative surface areas of different gut regions are probably unaffected by the exclusion of microvillae from surface-area estimates, however, as the density of microvillae is similar in all gut regions (personal observation). This limitation is not peculiar to our study; light microscopy cannot resolve features smaller than 0.5-1.0 micrometer. Histological sections were not made of *Thelepus* gut sections.

### 2.3. Absorptive accumulation determinations

The ratio of gut tissue substrate concentration : incubation solution concentration ( $R$ ) quantifies substrate accumulation over and above the concentration of the incubation solution and indirectly measures the density of transporter sites. We measured  $^{14}\text{C}$ -tagged D-glucose, L-methionine, L-tyrosine, L-aspartic acid, and L-glutamic acid (New England Nuclear, Boston, MA) taken up by tissue, and tissue fluid volume, to obtain tissue substrate concentration. Incubation solution substrate concentrations were estimated from samples.

The glucose incubation solution concentration was set at 500  $\mu\text{M}$ , approximately 5X greater than measured for *Parastichopus* gut fluid (Self et al., 1995). Tyrosine, aspartic and glutamic incubation solution concentrations were also set 5X higher than *Parastichopus* gut fluid concentrations at 50  $\mu\text{M}$ . Methionine concentration was set at 500  $\mu\text{M}$ , 100X higher than its measured concentration in *Parastichopus* gut fluid. The steeper methionine gradient pushes the

limits of the uptake system for this essential amino acid. A brief description of methods follow. For a more detailed description see Self et al. (1995) and Karasov & Diamond (1983).

Incubation solutions were prepared with artificial seawater (Sigma Chemical) then dispensed in 8-mL aliquots to 140mm X 16-mm diameter, flat-bottomed test tubes. Radio-labelled substrates were then added such that minimal tissue and solution counts were 100× greater than background.

One-centimeter long segments were cut from dissected guts, everted, then slipped onto a close-fitting glass rod 2 -7 mm in diameter and the length measured through an ocular micrometer. The rod-mounted sleeves were pre-incubated for 5 min in filtered seawater at ambient (10 - 14°C) seawater temperature, then transferred to the incubation solutions (also kept at 10 - 14 °C). During incubation the solution was agitated by stirring at 1,200 rpm with a stir-bar. The 20 min incubation was terminated by rinsing for 20s in 75 mL cold (0 °C) filtered seawater (also stirred at 1,200 rpm). After removing excess fluid with blotting paper, the sleeve was placed in a tared glass scintillation vial and weighed to obtain the wet mass. The segment was dried over-night at 55 °C then reweighed to obtain dry mass. Tissue fluid volume in microliters equals wet minus dry mass expressed in milligrams.

Tissue samples were solubilized (Soluene 350, Packard Instrument Co.), scintillation cocktail added, then acid-neutralized with acetic acid. Radioactivity was determined on a Beckman LS7800 liquid scintillation spectrometer with nominal window settings: <sup>3</sup>H channel 0-400, <sup>14</sup>C channel 400-670.

We calculated absorptive accumulation as the dimensionless ratio

$$R = \frac{\left(\frac{T/H}{s}\right)}{C}, \text{ where} \quad (1)$$

$T$ = substrate DPM associated with the tissue sample,  $H$  = substrate specific activity (DPM per nanomole),  $s$  = tissue fluid volume,  $C$  = incubation solution concentration.

We compared absorptive accumulation by anterior gut sections where digestive (Mayer et al., 1997) and nutrient uptake (Self et al., 1995) rates are highest. For this screening we assumed that the empirically determined values for *Parastichopus* in Self et al. (1995), such as rinse times, gut fluid substrate concentrations, incubation times, etc., applied to all species. Anomalous findings would direct future efforts toward those species and substrates.

#### 2.4. Statistical procedures

Inspection of the frequency distribution of *R* showed it to be log-normal and mean values linearly correlated with variances. Therefore statistical analysis was performed on log-transformed values and back-transformed geometric means reported. We concluded substrate accumulation had occurred if T-test (Sokal & Rohlf, 1969, pg 168) results inferred that mean ratios were significantly greater than one (i.e. tissue substrate concentrations exceeded incubation solution concentrations). Species, substrate and interaction effects were evaluated in a 5 x 6 (unevenly replicated) ANOVA. The within individual mean square proved to be the largest (0.26) and we used it to calculate conservative F-ratios.

We estimated reserve uptake capacity (*sensu* Diamond, 1991) of glucose and AA for *Parastichopus*. Reserve uptake capacity is the ratio of the gut's capacity to absorb substrate to its availability in the animal's sediment food. We estimated capacity from measured maximum absorption rates (Self et al., 1995) and intake of AA from enzymatically-available (i.e. digestible) concentration on sediments from *Parastichopus* feeding areas multiplied by sediment ingestion rate. Measurements of enzymatically-available D-glucose have not been done. We estimate digestible D-glucose to be 16% (Cowie, 1992) of total organic carbon measured in surface layer sediments nearby in Puget Sound (Mayer, 1994).

### 3. Results and discussion

#### 3.1. Digestive-tract and epithelial morphology

##### 3.1.1. Holothuroids

The digestive tracts of *Parastichopus* (Order Aspidochirotida) and *Molpadia* (Order Molpadida), are typical of those for holothuroids in their respective orders (Fig 1). The pharynx, esophagus, descending small intestine, ascending small intestine, large intestine, and cloaca are the major regions. The pharynx and cloaca were not included in this study, as they play minor roles in digestive-enzyme secretion and nutrient absorption (Feral & Massin, 1982).

Esophagus -- The degree of epithelial folding varies among species. Prominent longitudinal folds extend into the lumen of the anterior esophagus of *Parastichopus*, becoming smaller and more irregular in the posterior esophagus. In the esophagus of *Molpadia*, very shallow, angular, transverse folds extend around part of the circumference, while the remaining epithelium is smooth.

Stomach -- Regular, closely spaced folds extend prominently into the lumen of the anterior stomach of *Parastichopus*, and there is significantly less epithelial folding in the posterior stomach. Near the junction with the small intestine in *Parastichopus*, the size of epithelial folds increases.

Descending small intestine -- Prominent transverse folds of epithelium extend into the lumen of the descending small intestine of *Parastichopus*. The folds occlude most of the gut lumen in *Parastichopus*, where they are termed 'lamellae' (Fig 2, 3). The lamellae are arranged in an alternating pattern of larger and smaller folds, and are very closely spaced. They extend throughout the anterior and mid-regions of the descending small intestine. The epithelium of the posterior region is highly folded, but irregularly, and not in the form of lamellae. The epithelium is relatively smooth throughout the descending small intestine of *Molpadia*, both in cross and longitudinal sections (Fig 4).

Ascending small intestine -- In the ascending small intestine of *Parastichopus* there are small, irregular, shallow folds that decrease in size posteriorly throughout the gut region. The degree of epithelial folding increases near the junction with the large intestine. The epithelium of this gut region is very smooth in *Molpadia*.

Large intestine -- The epithelium forms broad, shallow, irregular folds in the anterior region of the large intestine of *Parastichopus*. The mid-region of the large intestine of *Parastichopus* is

relatively smooth (Fig 5), and large, irregularly shaped, sparse folds are present in the posterior large intestine. The epithelium in the large intestine of *Molpadia* is more highly folded than that in the small intestine (Fig 6). Folds are present in both longitudinal and cross sections, are not closely spaced, and are irregular in form and distribution. They increase in size toward the posterior end of the intestine.

Cell types -- Our classification of holothurian epithelial cell types are guided by Feral & Massin (1982) and Smiley (1994). Cells secreting digestive enzymes contain dark-staining granules, possibly zymogen-secreting. In *Parastichopus* they occurred only in the slides of the folds from the anterior descending intestine. However, they look quite different with apical, instead of central granules. They occur in clusters (1-4 cells) and are less than 10% of the total cell number. Their zymogen-secreting character has not been demonstrated. We were not able to identify any zymogen-secreting cells in *Molpadia*. A brushborder and/or cilia are too small to see in our sections. The consensus view among cell morphologists seems to be that nutrient absorption can occur all along the holothurian gut.

### 3.1.2. *Brisaster latifrons*

The digestive tract consists of a main gut canal divided into an esophagus, small intestine, large intestine, rectum, and two large caeca connecting to the main gut canal: the gastric caecum and the intestinal caecum (Fig 7). The esophagus and rectum are narrow, while the small and large intestines are relatively wide. The gastric caecum, a large sac-like structure, connects to the main gut canal at the junction of the esophagus and the small intestine, and the intestinal caecum is flattened, connecting to the main gut canal at about the midpoint of the large intestine. Part of the inner border of the small intestine is constricted to form a slender tube termed the siphon, which extends diagonally across the body cavity from near the posterior end of the esophagus to about the midpoint of the small intestine.

Esophagus -- The esophagus can be divided into three regions: a relatively wide, short anterior region connecting to the mouth, a narrow middle region extending to the anterior end of the siphon, and a narrow posterior region extending to the anterior end of the small intestine. With the

exception of the middle region, where the epithelium is highly folded on one side of the lumen, the epithelium is relatively smooth throughout the esophagus.

Small intestine -- A characteristic arrangement of smooth and ridged epithelium lines the anterior two-thirds of the small intestine. The gut wall on the aboral side is thrown into a series of transverse ridges extending along 25% of the gut perimeter (Fig 8). The epithelium is folded within these ridges to create closely packed, transverse, epithelial ridges. The gut wall and epithelium are both smooth around the entire gut circumference of the posterior third of the small intestine.

Large intestine and rectum -- The epithelium on one side of the lumen is relatively smooth, while that on the other invaginates at wide intervals to create shallow clefts (Fig 9). Throughout most of the posterior half of the large intestine, transverse folds of the gut wall extend along half of the gut perimeter, and broad, transverse folds of epithelium are located within the gut-wall folds. The gut wall and epithelium are smooth in approximately the most posterior 15% of the large intestine. The epithelium of the rectum forms sparse longitudinal folds that are relatively broad and irregularly shaped.

Caeca -- The epithelium of the gastric caecum is thrown into numerous, shallow, very closely packed folds (Fig 10), which are present in both longitudinal and cross sections. The epithelium of the intestinal caecum is relatively smooth, forming only small, irregularly distributed folds.

Cell types -- We were not able to identify potential zymogen-secreting cells although they have been found in the stomach (our small intestines) of the intertidal, sandy-beach dweller, *Echinocardium cordatum* (DeRidder & Jangoux, 1993).

### 3.1.3. *Arenicola marina*

The digestive tract is divided into the following regions: the esophagus, esophageal caeca, cardiac stomach, post-cardiac stomach, anterior intestine, and posterior intestine (Fig 11). The eversible part of the esophagus is not included in this study. Using the criteria of Penry & Jumars (1990) for classifying digestive tracts, *Arenicola* is a species with four gut compartments: an anatomically distinct foregut (esophagus), two anatomically distinct, non-muscular subregions of the midgut (cardiac stomach, post-cardiac stomach), and a hindgut (posterior intestine). The

anterior intestine is morphologically similar to the post-cardiac stomach. *Arenicola* can be treated approximately as a three-compartment gut, as the relative length of the cardiac stomach is very small.

Esophagus -- The non-everting portion of the esophagus consists of three histologically distinct regions: an anterior jugular region, a middle muscular region, and a posterior glandular region. The epithelium of the muscular region forms closely packed, relatively shallow, longitudinal folds. Closely spaced longitudinal folds are also present throughout most of the glandular region, where they are broader and extend farther into the lumen than in the muscular region. Approaching the connections with the caeca, epithelial folds are smaller and are present in both longitudinal and cross sections. The epithelium is smooth at the connections with the caeca.

Esophageal caeca -- The epithelium of the caeca forms broad, longitudinal folds protruding prominently into the lumen and extending throughout the length of the caeca (Fig 12). The folds are arranged in an alternating pattern of shorter and taller folds, and are not closely packed.

Stomach -- The stomach includes a very short, anterior cardiac region and a long post-cardiac region. The epithelium of the cardiac stomach is organized into shallow, tightly packed, longitudinal ridges. The outer gut wall of the post-cardiac stomach is in the form of closely packed, outfolded pouches that decrease in size posteriorly. The epithelium within each pouch is highly folded, forming dense, large, closely packed loops that occupy most of the lumen within the pouches (Fig 13).

Intestine -- There is a gradual morphological transition between the post-cardiac stomach and the anterior intestine. For this study, the boundary was placed at segment 14, where there is a conspicuous decrease in gut diameter. As in the post-cardiac stomach, the gut wall of the anterior intestine forms pouches that contain prominent, closely packed folds of epithelium. The pouches are more shallow than those of the post-cardiac stomach. The outer wall of the posterior intestine is smooth. In the anterior region of the posterior intestine, the epithelium forms long, transverse loops extending into the lumen on opposite sides of the gut. There are regular, shallow invaginations of epithelium along the length of these loops. The degree of epithelial folding

decreases posteriorly within the anterior two-thirds of the posterior intestine (Fig 14). The epithelium of the posterior third, which is cuticle lined, forms shallow, closely packed transverse ridges that increase in height posteriorly. The ridges also become more jagged posteriorly.

Cell types -- Potential zymogen-secreting cells, based on shape, size, arrangement and staining properties of the granules can be detected only in the stomach (post-cardiac). Three to four cell clusters per 20 cell patches occur. Over the whole section their fraction is less than 10%. Michel's (1988) review of TEM studies conclude that absorptive and secretory cells co-occur in the stomach and anterior intestines of *Arenicola*.

#### 3.1.4. *Amphitrite johnstoni* (and *Thelepus crispus*)

The digestive tracts of *Amphitrite* and *Thelepus* are similar in all respects and consist of the esophagus, fore-stomach, hind-stomach, anterior intestine, and posterior intestine (Fig 15). Using the criteria of Penry & Jumars (1990) for classifying digestive tracts, *Amphitrite* is a species with five gut compartments: an anatomically distinct foregut (esophagus), a midgut subdivided into an anterior, non-muscular subregion (fore-stomach) and a posterior, muscular region (hind-stomach), and a hindgut divided into anatomically distinct anterior (anterior intestine) and posterior (posterior intestine) subregions.

Esophagus -- The epithelium forms transverse folds anteriorly and longitudinal folds posteriorly. The longitudinal folds extend prominently into the lumen and are very closely packed.

Fore-stomach -- The epithelium is highly folded (Fig 16). In much of the fore-stomach, the epithelium forms dense, tightly packed folds that extend prominently into the lumen. In regions of less extensive folding, folds are shallow, broad, and closely spaced, and appear to be associated with mucus secretion. Folds appear to be primarily longitudinal.

Hind-stomach -- The epithelium is very smooth throughout the region (Fig 17). There are only scattered, very shallow invaginations of epithelium.

Anterior intestine -- Other than the folds bounding the ventral ciliary gutter, the epithelium is relatively smooth longitudinally, forming only scattered folds. The epithelium does form deep, narrow, regularly spaced, transverse invaginations.

Posterior intestine -- In addition to the folds of the ventral ciliary gutter, there are prominent, closely spaced longitudinal folds of epithelium that protrude into the lumen around the gut circumference. These folds extend throughout the posterior intestine.

Cell types -- Gomori's stain does not seem to stain secretory granules as we cannot find the granules described by Michel et al. (1984) in the related *Terebellides stroemi*. In an early study (Dales, 1955), some potentially zymogen secreting cells were observed in the fore stomach and much more in the fore intestines. However, no quantitative data are available.

### 3.2. Epithelial surface area

Several gut regions have particularly highly folded epithelia. High values are produced by the transverse lamellae in the esophagus, stomach and descending small intestine of *Parastichopus* (Figs 2, 3), the dense, small folds in the gastric caecum of *Brisaster* (Fig 10), and prominent folds in the esophageal caeca (Fig 12) and post-cardiac stomach (Fig 13) of *Arenicola*. The fore-stomach (Fig 16) and anterior intestine of *Amphitrite* have moderately high values produced by a moderate degree of epithelial folding. The epithelium is relatively smooth throughout the entire digestive tracts of *Molpadia* (Fig 4,6), posterior section of *Parastichopus* (Fig 5) and non-caecum sections of *Brisaster* (Fig 9).

The ratio of epithelial surface area to surface area of a cylinder of equivalent length and radius (GSA : CSA) ranges among gut regions from 1 to 14 (Table 1, Fig 18). This dimensionless parameter reflects the degree of epithelial folding, with high values indicating a convoluted epithelium and low values a relatively smooth epithelium. The distribution of epithelial folding among digestive-tract regions has a similar pattern in three of the five observed species, *Arenicola*, *Parastichopus* and *Brisaster*; the epithelium is most highly folded in an anterior region and relatively smooth in posterior regions (Fig 18). *Amphitrite* has a somewhat intermediate pattern with slightly higher folding in the intestine region. The exception to the pattern is *Molpadia*; its epithelium is most highly folded in the large intestine (Fig 6), a posterior gut region, and is relatively smooth elsewhere (Table 1, Fig 4, 18).

The arrangement of a highly folded anterior region followed by a relatively long but smooth posterior region (Fig 18) suggests that a comparatively large volume of enzyme is needed to initiate rapid hydrolysis of the limited food substrate. Enzyme secretion in holothuroids takes place primarily in the stomach and anterior region of the descending small intestine, despite a functional overlap among some gut regions (Bai, 1978; Fish, 1967a, b; Rosati, 1968, 1970; Farmanfarmaian, 1969a, b; Massin, 1980; Filimonova & Tokin, 1980). Digestive-enzyme secretion in *Brisaster* probably takes place primarily in the gastric caecum and small intestine, as these two gut regions are the primary sites of digestive-enzyme secretion in the closely related *E. cordatum* (DeRidder & Jangoux, 1993). This need for enzyme secretion would only be exacerbated if, as expected, enzymes fall prey to inactivation in organically complex sediments. These secretory regions are also heavily muscularized. The close spacing of folds in the descending small intestine of *Parastichopus*, the post-cardiac stomach of *Arenicola*, the fore-stomach of *Amphitrite*, and the small intestine of *Brisaster* appears to exclude particulate matter from the spaces between adjacent folds. This geometry results in a large reservoir of fluid that we suspect is injected by muscular contraction, accordion like, into ingested material. Epithelial folding increases the number of epithelial cells per unit of gut length, and in all observed species but *Molpadia*, the gut region with the largest, if not equal surface area amplification (*Amphitrite*), is thought to be a primary site of digestive-enzyme secretion.

The gastric caeca of *Brisaster* and the esophageal caeca of *Arenicola* may contribute to enzyme supply and mixing in the small intestine and post-cardiac stomach, respectively, by supplying fluid to these regions. Fluid has been observed flowing into the main gut channel from the caeca of *Arenicola* (Kermack, 1955; personal observation) and the gastric caecum of *Echinocardium cordatum* (DeRidder & Jangoux, 1993), an irregular echinoid closely resembling *Brisaster* in feeding biology and gut morphology. The caeca of these species are generally filled with fluid, and there is no evidence that sediment enters the caeca of *Arenicola* (C. Plante, personal communication; personal observation), *Brisaster* (personal observation), or *E. cordatum* (DeRidder & Jangoux, 1993). Secretion of digestive enzymes in *Arenicola* takes place primarily

in the post-cardiac stomach and the esophageal caeca (Kermack, 1955; Longbottom, 1970; Bamford & Stewart, 1973a, b; Kaganovskaya, 1976, 1978a, 1978b, 1983; Mayer, unpublished). Digestive-enzyme secretion and extracellular digestion in *Amphitrite* are concentrated in the fore-stomach and the anterior region of the anterior intestine (Dales, 1955; Mayer, unpublished).

### 3.3. *Transporter density*

On exposure of gut sleeves to a substrate solution, in which concentration is very much greater than apparent  $K_m$ , our expectation is for the carrier-mediated uptake system to saturate prematurely. The steep concentration gradient allows diffusive influx to continue at a rapid rate until tissue concentration equals the external concentration of the incubation solution.  $R$  values (Eqn 1) near or equal to unity result. Alternatively, ratios greater than one imply that the transporter system stands ready to accommodate short-term concentration spikes by accelerating uptake. The degree of accumulation against that gradient, as measured by the magnitude of  $R$ , roughly approximates the specie's allocation of limited luminal space to obtaining that substrate.

We know most about uptake kinetics in *Parastichopus* guts (Self et al., 1995) so we summarize aspects of its gut physiology, then look at results for absorptive accumulation by *Parastichopus* and compare that with accumulation by other species.

#### 3.3.1 *Parastichopus*

Using a scale developed for vertebrates (Karasov, 1988), *Parastichopus* scores as a herbivore with values of glucose : amino acid uptake well above unity. On this scale omnivores and carnivores score lower because glucose uptake decreases, rather than AA uptake increasing. All taxa require protein hence the constancy of AA uptake. Presumably, the high carbohydrate diet of herbivores results in more glucose transporters allotted to the fixed luminal surface area of the gut.

The well-documented atrophy (winter) then regrowth (spring) of the gut (Swan, 1961; Fankboner & Cameron, 1985; Self et al., 1995), synchronized with the phytoplankton bust and bloom cycle, suggest an anatomic adaptation to the seasonal availability of food as observed in vertebrates such as wild rabbits (Sibly et al., 1990), prairie voles (Hammond, 1993) and deer

(Weckerly, 1989). What remains unknown is whether the spring regrowth is an endogenous rhythm cued by an environmental factor such as day-length, water temperature or diet quality, or merely the cessation of a starvation condition. Further, in our experiments where protein and carbohydrate content of the diet were varied we did not detect specific modulation of L-aspartic acid or D-glucose uptake (unpublished data). Our working hypothesis is that transporter density and distribution along the gut of *Parastichopus* is genetically fixed and not modulated by diet. Rather, the evolved response to predictable variation in diet appears to be increasing the size of the absorbing organ.

*Parastichopus* capacity : intake (C/I) ratios (Table 2) varied by 3 orders of magnitude across substrates. The value for glucose is well within expected limits for a non-essential nutrient (Diamond, 1991) with a reserve capacity capable of responding to twice the normal intake of glucose in the *Parastichopus* diet. Values for tyrosine, aspartic acid and glutamic acid are unexpectedly high for non-essential AA and comparable to the hyperessential nutrient arginine in cats (Diamond & Hammond, 1992). Extremely high methionine C/I values reflect the paucity of this nutrient in *Parastichopus*'s surface-sediment diet coupled with a high  $J_{\max}$ . Values for  $J_{\max}$  (Table 2) represent the central tendency for the whole gut, not only anterior gut sections. A sense of how a high  $J_{\max}$  is achieved can be gained by realizing that methionine was the only substrate for which *Parastichopus* maintained transporters in the hindgut (Self et al., 1995) in spite of a measured (and presumed normal) luminal concentration of less than 1  $\mu\text{M}$ .

*Parastichopus* did not generate unusually high absorptive accumulation ratios (Table 3). Ratios for glucose and the non-essential AA aspartic acid and glutamic acid were not significantly greater than unity. Ratios for methionine and tyrosine were significantly greater than one. However, if we apply the Bonferroni  $F$  procedure (Huitema, 1980) to account for the fact that 30  $T$ -tests were performed, then to obtain an overall  $P$  level of 0.05, individual  $T$ -tests are set at an experimentwise level of 0.002. Using this more conservative level only tyrosine was accumulated by *Parastichopus* gut sleeves against the preset 50 $\mu\text{M}$  concentration gradient.

The difference in  $R$  between tyrosine and methionine (for *Parastichopus* as well as other species) is in part due to the concentrations at which the measurements were performed -- tyrosine at 50 $\mu$ M and methionine at 500 $\mu$ M. We know that in *Parastichopus*  $J_{\max}$  for methionine is 20x greater than  $J_{\max}$  for tyrosine (Self et al., 1995). Thus at equal concentrations we would expect methionine accumulation to exceed tyrosine. Bamford & Stewart (1973a) have shown the concentration effect on accumulation of L-alanine by *Arenicola* guts over a 100 $\mu$ M - 1000 $\mu$ M concentration range. The technique has also been applied to uptake across the body-wall of marine invertebrates (Wright, 1988). Higher values of  $R$  were measured at lower substrate concentrations, i.e. before the onset of carrier saturation and subsequent slowing of uptake. Applying the Bamford & Stewart relationship to our data, we estimate that  $R_{\text{meth}}$  at 50 $\mu$ M would be 25% higher than its current value of 1.8 at 500 $\mu$ M, or 2.3, closer to the  $R_{\text{tyr}}$  value of 3.0.

Seeking a plausible explanation for *Parastichopus*'s propensity for methionine uptake, led us to the literature on how marine invertebrates maintain solute balance between cellular spaces and the external environment. For example, in the congeneric species *P. tremulus* fluid spaces of muscle tissue are maintained in osmotic balance with the coelomic fluid and external seawater by substituting betaine and free AA for ionic components (Robertson, 1980). The high ionic content of the external medium, if duplicated in cellular spaces, would destabilize functional properties of macromolecules (Hochachka & Somero, 1984). An essential feature of organic osmolytes is their limited reactivity with protein enzymes (Clark, 1985) at physiological pH (King, 1988). Non-reactivity with another class of protein macromolecules, nutrient transporters, may prevent aggressive osmolyte accumulation from the diet. The sulfur AA taurine is often one of the dominant free AA constituents of marine invertebrate tissue (Awapara, 1962; Bishop et al., 1983; Yancey et al., 1982) and derives its innocuous effect on macromolecules from its structural resemblance to ammonium sulfate (Clark & Zounes, 1977). An alternative pathway for accumulation of taurine by *Parastichopus* may be through uptake of the sulfur AA methionine from the diet followed by conversion to taurine as demonstrated in *Arenicola cristata* (Abbott & Awapara, 1960).

### 3.3.2. Species comparisons

D-glucose ratios were less than or equal to one in all species (Table 3, Fig 19A) when the guts were presented with a 500 $\mu$ M gradient. We know that D-glucose uptake is carrier-mediated in *Parastichopus* and the

concentration in the gut fluid of anterior gut sections can be about 500 $\mu$ M, well below the estimated saturating concentration of 12,000  $\mu$ M (Self et al., 1995). At 500 $\mu$ M passive and carrier-mediated uptake are, respectively, 24% and 76% of total uptake. A possible mechanism is that passive out-flux offsets carrier-mediated influx and D-glucose does not accumulate much above the concentration found in the gut lumen.

D-glucose ratios significantly less than one in *Brisaster* and *Arenicola* could be caused by a mis-matching of *Parastichopus*'s incubation time to these species. Alternatively, these species have lower inherent cell membrane permeability and transporter density, or higher cytoplasmic D-glucose concentration compared to *Parastichopus* gut cells. A minimum two minute incubation time was required for equilibration between the bulk incubation solution and fluid layers adjacent to *Parastichopus* gut wall (Self et al., 1995). This minimum time is a function of luminal surface area and turbulent mixing of the incubation solution. The 20 minute incubation time used here, 10X greater, under identical mixing conditions, should be more than adequate to offset any differences in luminal surface area between species. Thus, ratios significantly less than one are more likely due to structural or functional differences related to permeability, transporter density or cellular D-glucose concentration. Lower permeability suggests a structural barrier to diffusion, perhaps in the form of tighter cell junctions, such that longer incubation times would be required for equilibration. Greater internal D-glucose concentration lessens or possibly negates the intended 500 $\mu$ M gradient and thus direction of net D-glucose flux. Lower transporter density would lead to early saturation at a lower substrate concentration and uptake rate. Whichever mechanism obtains, clearly, D-glucose is not aggressively amassed by the guts of these deposit-feeders implying that its supply in the diet is plentiful and predictable. This result contrasts with amino acid nitrogen sources which tended to be accumulated.

In general mean AA ratios tended to be greater than one, varying by an order of magnitude across species and substrates (Table 3, Fig 19A). For aspartic and glutamic acid only *Molpadia* and *Thelepus* ratios were significantly greater than one at  $P < 0.001$ . Methionine and tyrosine ratios tended to be higher than the acidic AA. The exception being *Brisaster*'s methionine ratio which was not significantly greater than one. Again applying the more conservative Bonferroni  $F$  procedure (Huitema, 1980) and setting the experimentwise level at  $P = 0.002$ , only *Molpadia* and the unusually high methionine and tyrosine ratios of the terebellid polychaetes (Table 3, Fig 19A) are acceptable.

The terebellid polychaete species had small gut diameters and consequently lower average tissue fluid volumes (Fig 20A). Gut tissue fluid volume is inversely correlated with  $R$  (Eqn 1). We explored the possibility that high ratios for the terebellids were driven, not by a greater transporter density, but by lower tissue fluid volumes. Perhaps transporter density and therefore uptake remain constant across species but since terebellid fluid volumes were small, their tissue concentrations are artificially boosted. Recalculating substrate tissue concentration in terms of luminal surface area (Fig 19B) or dry mass (Fig 19C), instead of fluid volume, corroborate the initial pattern of high uptake and retention of methionine and tyrosine by *Thelepus* and *Amphitrite* guts.

Exceptionally high ratios are caused by sustained high uptake rates which in turn are driven by a high number of transporter sites allocated to the fixed area of the luminal surface. In spite of relatively low luminal surface areas (Fig 20B), primarily due to small gut diameters, the terebellids accumulated methionine and tyrosine (Fig 19A) implying spatially dense concentrations of these transporter sites. Corroborating anomalous uptake of methionine and tyrosine by the terebellids ANOVA results of the Fig 19A data showed significant statistical interaction ( $SS = 9.3$ ,  $df = 20 \text{ \& } 337$ ,  $MS = 0.47$ ,  $F = 1.8$ ,  $0.01 < P < 0.03$ ) between species and substrate confounding unambiguous interpretation of significant species and substrate factor effects. In part, nonlinear (nonadditive) distribution of means among species and substrates (i.e. the upward spikes of terebellid methionine and tyrosine geometric mean values) caused the statistical interaction. Our attempts to find a transformation which would increase model additivity failed. We conclude that the terebellids indeed allot an anomalous (compared to other species) proportion of their limited gut luminal surface area to the absorption of methionine and tyrosine.

Investigating the contribution of low molecular weight nitrogen compounds to invertebrate intracellular osmotic activity, Clark (1968) found an “anomalously” high amino acid nitrogen content in the body wall and coelomic fluid of *Thelepus* among fourteen tidal and subtidal polychaete species (including two other terebellid species) from the area near Friday Harbor Laboratories. No apparent phylogenetic or environmental trend emerged. However, she noted the toughness of *Thelepus*'s tube of secreted material, compared to the fragile, sandy construction of the other terebellid species. Perhaps *Thelepus*'s tube is less permeable and subject to greater solute disequilibria with external saltwater. The burrow lining of the terebellid polychaete *Amphitrite*

*ornata* reduced diffusion of small inorganic solutes by 88% (Aller, 1983) contributing to buildup of metabolites (Aller & Yingst, 1978) in the absence of increased irrigation rates (Aller, 1980; Woodin & Marinelli, 1991). A reasonable hypothesis for anomalous accumulation of methionine from their diet by intertidal terebellids is to offset osmotic imbalance between tube-water and cellular spaces by conversion to the well known osmolyte taurine, as demonstrated in *Arenicola crista* (Abbot & Awapara, 1960).

#### 3.4. Gut geometry, enzyme secretion and nutrient uptake

Induction of enzyme secretion requires signals from the ingested food slurry to the enzyme-secreting apparatus; evidence for this capability is provided by studies of the intestines of holothuroids (Garcia-Arrars et al., 1991) and polychaetes (Baratte et al., 1990; Dhainaut-Courtois et al., 1985) demonstrating the presence of neurotransmitters that are thought to play an important role in regulating intestinal physiology. In this scenario, the epithelial folding provides a volumetrically significant capability for inducible digestive enzyme production that is, in a topological sense, mixed in with the luminal fluids. Significantly, this topological mixing is with the digestive fluids and not with the sedimentary substrate, which we did not find among the folds.

Evidence that fluid mixing takes place in regions of high epithelial folding is provided by observations of loosely compacted sediment in these regions. In deposit-feeding holothuroids, sediment is least compacted in the descending small intestine, with sediment becoming increasingly compacted posteriorly along the length of the digestive tract (Massin, 1980). The post-cardiac stomach of *Arenicola* contains loosely compacted, fluidized sediment (personal observation) with a high fluid content (Kermack, 1955; Plante & Mayer, 1994). Although sediment is not particularly loosely compacted in the fore-stomach of *Amphitrite*, fluid may be mixed by the gut musculature. Muscular contractions of the esophagus are peristaltic, while those in the anterior intestine are anti-peristaltic (Dales, 1955). Contractions in opposite directions on either side of the fore- and hind-stomachs may mix fluid in those regions and in the anterior intestine.

The open question that remains is whether the mixing is sufficient to meet the assumptions of a PFR (complete radial mixing) or to exceed them, producing significant axial mixing along with some of the characteristics of a CSTR. With x-radiography, Penry (1989) demonstrated a lack of either axial or radial particle mixing in *Parastichopus*, and we doubt that axial mixing in this species is sufficient to alter the plug-

flow assumptions significantly. The axial scale of (anti)peristaltic mixing of fluids in a long, tubular gut is unlikely to exceed one or a few gut diameters (e.g., Melville et al., 1975; Macagno & Christensen, 1981). Conversely, (Penry, 1989) documented substantial axial mixing of particles in the gut of the ampharetid polychaete *Amphicteis scaphobranchiata*, whose gut construction is roughly similar to those of the two terebellids examined here in terms of having widened, muscular sections that may behave more like CSTRs. The details and value of such mixing remain to be worked out.

The radial mixing assumed in all three ideal reactor types is important for both hydrolysis and absorption but doubly so for speeding hydrolysis. If enzymes were secreted at the epithelial lining and diffused inward by molecular diffusion alone, a moving front of nonzero enzyme concentration would ensue, and products would form initially in a cylindrical section of lumen next to the wall. The problem with this geometry in product concentration is that it will drive molecular diffusion of products inward as well as outward (Fig. 1?? **[Should I draw a figure?]**) Rapid initial mixing of enzymes through the gut lumen, on the other hand, will produce molecular diffusion only in the favorable direction toward the wall assuming either passive or active uptake at the wall. Continued radial mixing during absorption will enhance the rate of outward radial transport of products to the extent that it steepens the near-wall concentration gradient but will not alter the direction of transport.

High AA transporter density generally did not co-occur with increased epithelial surface area (Table 1; Fig 21). Absorption of AA in holothuroids took place in all gut sections (not measured in the esophagus), but tended to be highest in fore- and midgut regions. Uptake rates of four amino acids by the gut epithelium of *Parastichopus* are generally greatest in the ascending small intestine (Self et al., 1995). Results in this work replicate this past finding. Amino acid transporter sites of the ascending small intestines of *Parastichopus* act to double the tissue concentration of L-aspartic acid, L-glutamic acid and L-methionine over that expected by diffusion alone (Table 1, Fig 21). Some absorption in holothuroids may also occur in the descending small intestine (Rosati, 1968, 1970; Farmanfarmanian, 1969b, Self et al., 1995) and confirmed by our results for *Molpadia*. The anomalous trend of decreasing AA accumulation moving posteriorward in *Molpadia* is ambiguous because the 95% confidence limits about the medians of the second and third gut sections (stomach

& descending small intestines and ascending small intestines) overlap (Table 1). But clearly, AA transporter density is lowest in the large intestines offsetting its increased epithelial surface area.

Amino acid absorption is lowest in the esophagus, then essentially uniform throughout the remainder of the gut of *Brisaster*. This result contrasts with the regional specialization of *Echinocardium cordatum*'s gut based on ultrastructure and histochemical analysis (DeRidder & Jangoux, 1993) -- a mucous-secreting esophagus, digestive caecum and stomach (our small intestines) and an absorptive large intestine and intestinal caecum. Our direct measurements of AA accumulation do not indicate absorptive specialization in any single gut section. The difference in sediment food of these urchins, fine, organic-poor mud for *Brisaster* and organic floc-sand mixture for *E. cordatum* could account for the difference between study conclusions.

Amino acid absorption in *Arenicola* takes place primarily in the stomach and intestine (Fig 21). The esophageal caeca of *Arenicola* are also sites of absorption, but much reduced, suggesting that enzyme secretion is the primary function of the enhanced epithelial surface area. The highly folded stomach is a site of enhanced L-methionine, but not L-aspartic acid or L-glutamic acid absorption.

Exceptionally high methionine accumulation by the fore- and midgut sections of *Amphitrite*, indicative of enhanced transporter density for this AA, distinguish this species from all others (Fig 21). The west coast terebellid *Thelepus crispus* also shows this trend (Table 3). The longitudinal distribution of epithelial surface area varies by approximately 2x and is highest in the anterior intestine. This gut section also had the highest combined AA accumulation. Pioneering work (Dales, 1955) indicated that absorption (of iron saccharate) takes place in the anterior intestine and in the anterior part of the posterior intestine, but not in the fore-stomach. This work confirms the former, but concludes that exceedingly high uptake of some nutrients can also take place in the fore-stomach.

This work shows that AA absorptive potential and epithelial surface area amplification vary on the order of 10x across gut sections and species (Fig 21; Fig 22). In contrast, variables related to the digestive process, e.g. the 1,000x variation in summed protein concentration and 10,000x variation of summed protease activity clearly shows that digestive potential rather than absorptive potential or gut geometry differentiates species (Fig 22; Mayer et al., 1997). Phyletic differences are a possible explanation. Annelids generally have a more advanced circulatory systems than echinoderms which speeds the transfer of nutrients away from gut cells

allowing the “upstream” digestive and absorptive reactions to proceed at higher rates. Alternatively, intertidal and nearshore species generally feed on sediments and organic flocs with higher organic content. Hence the nutrient load is greater than for offshore species making it worthwhile, and perhaps necessary in competition with meiofaunal and micro-organisms, to deliver a high quantity, and/or quality, of enzyme to the gut lumen to rapidly hydrolyze what is still a dilute substrate. Physical variation in the intertidal environment, on daily as well as seasonal time scales (water temperature, salinity, solute concentration, desiccation), challenges successful species to adapt in different ways than subtidal species. Maintenance of an appropriate intra-cellular ionic environment, alluded to earlier as an explanation for enhanced L-methionine absorptive potential in terebellid polychaetes, is one possibility. Are other amino acids, deemed “essential” in insects and higher animals, e.g. arginine, phenylalanine and leucine, also in high demand by intertidal and subtidal species? Experiments could be organized to test these hypotheses grouping species by phyla and/or environment. For example, the absorptive and digestive potential of the irregular heart urchins *Echinocardium cordatum* and *Brisaster latifrons*, from intertidal sand and neritic mud environments, respectively, could be assessed with consistent and quantifiable methods.

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**Table 1.**

Epithelial surface areas measured from histological sections and compared to surface areas of cylinders of equal length and radius. Median accumulation (tissue concentration : incubation solution concentration) of essential (L-methionine) and non-essential (L-aspartic and L-glutamic acid) amino acids and 95% confidence limits. N = number of individuals.

Species	Gut Section	Gut Length (mm)	Gut Radius (mm)	Gut Surface Area (mm <sup>2</sup> )	Equivalent Surface Area of a Cylinder (mm <sup>2</sup> )	$\frac{\text{Gut Surface Area}}{\text{Equivalent Cylinder Surface Area}}$	Md Asp & Glut Accum	95%CL or Range	N (ind)	Md Meth Accum	95% CL or Range	N (ind)
<i>Amphitrite</i>	Esoph	6.9	0.5	48.3	21.7	2.2	-	-	-	-	-	-
	Fore St	21.1	2.9	812.4	384.5	2.1	1.5	1.3 - 2.4	6	11.9	10 - 18	5
	Hind St	19.6	1.5	203.8	184.7	1.1	.5	0.3 - 1.2	6	6.9	4.2 - 9.1	5
	Ant Int	97.8	1.5	2983.0	891.0	3.3	3.4	2.7 - 9.5	6	16.7	11 - 21	6
	Post Int	49.2	0.8	472.3	247.3	1.9	-	-	-	-	-	-
<i>Arenicola</i>	Esoph	20.6	1.3	271.9	168.3	1.6	3.1	0.1 - 6.6	5	1.4	0.6 - 5.9	4
	Esoph Caeca	12.7	1.3	1036.0	103.7	10.0	0.3	0.2 - 0.5	6	0.5	0.5 - 0.6	2
	Stom	42.3	1.9	3934.0	505.0	7.8	1.4	1.2 - 3.8	5	5.4	2.4 - 8.3	5
	Ant Int	8.5	1.2	204.0	64.1	3.2	3.8	2.7 - 6.7	6	3.2	3.1 - 6.7	5
	Post Int	29.6	1.8	748.9	325.5	2.3	-	-	-	-	-	-
<i>Parastichopus</i>	Esoph	27	1.0	799.2	161.2	5.0	-	-	-	-	-	-
	Stomach & Des Sm Int	128	2.1	9342.0	1689.0	5.5	1.0	0.6 - 1.2	5	1.3	0.9 - 1.6	4
	Asc Sm Int	120	2.0	1594.4	1508.0	1.1	2.4	1.5 - 5.1	4	3.0	1.8 - 4.3	4
	Lg Int	190	1.8	2385.5	2089.2	1.1	2.2	1.2 - 2.5	5	0.6	0.3 - 1.9	4
<i>Brisaster</i>	Esoph	26.4	2.1	385.4	348.3	1.1	0.6	0.3 - 1.1	6	0.6	0.2 - 1.7	3
	Gastric Caecum	16.9	2.9	4428.0	307.9	14.4	2.9	1.7 - 3.7	4	1.7	1.6 - 1.8	2
	Sm Int	69.2	1.7	1107.0	717.4	1.5	2.7	1.4 - 3.8	8	4.2	1.5 - 4.7	5
	Lg Int	78.2	2.8	1376.3	1375.8	1.0	1.9	0.7 - 5.2	9	2.7	0.6 - 4.0	6
	Intestinal Caecum	11.6	0.9	103.2	65.6	1.6	4.6	3.9 - 13.7	3	3.5	2.2 - 4.7	2
<i>Molpadia</i>	Esoph	21	1.1	144.9	145.1	1.0	-	-	-	-	-	-
	Stomach & Des Sm Int	67	1.7	917.9	715.7	1.3	4.3	1.8 - 6.9	10	3.9	0.5 - 5.2	7
	Asc Sm Int	63	1.6	781.2	633.4	1.2	2.5	1.6 - 3.6	9	2.2	1.1 - 3.5	7
	Lg Int	80	2.8	2360.0	1407.4	1.7	0.8	0.7 - 1.0	9	0.5	0.3 - 0.6	7

**Table 2**

Nutrient uptake capacity : intake ratio for *Parastichopus californicus*. Gut length is 70 cm and sediment ingestion rate is 21 gdw<sup>t</sup> day<sup>-1</sup> (Self & Jumars, unpublished; Yingst, 1982). Amino acid bioavailable concentration is enzyme hydrolyzable fraction (Mayer et al., 1995). D-glucose bioavailable concentration estimated from Mayer (1994) and Cowie (1992).

Substrate	$J_{max}$ (nmol cm <sup>-1</sup> min <sup>-1</sup> )	Uptake Capacity (μmole day <sup>-1</sup> )	Bioavailable concentration (μmole gdw <sup>t</sup> <sup>-1</sup> )	Intake (μmole day <sup>-1</sup> )	Capacity/Intake
D-glucose	2.6	260	3.7 - 6.7	78 - 140	2 - 3
L-aspartic acid	0.54	54	0.079	1.7	32
L-glutamic acid	0.40	40	0.116	2.4	17
L-tyrosine	0.10	10	0.009	0.19	53
L-methionine	2.37	240	0.011	0.23	1043

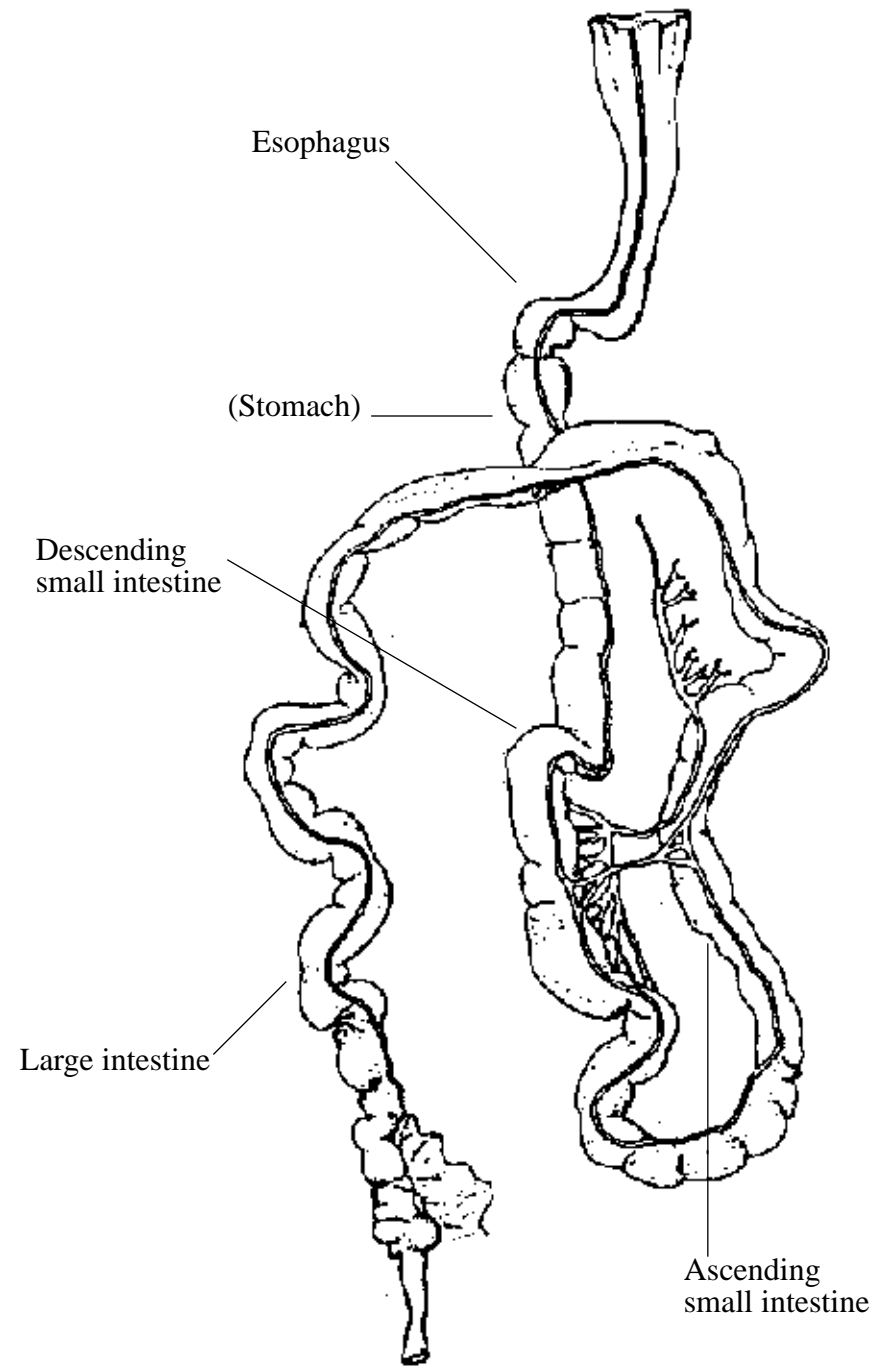
**Table 3**

Species by substrate (concentration gradient in parentheses) T-test results. μM: micromoles L<sup>-1</sup>;  $R$  = geometric mean substrate tissue concentration : incubation solution concentration ratio;  $n$  = number of anterior gut sleeves;  $P$  = T-test one-tail probability of rejecting the null hypothesis that  $R = 1.0$ . ns:  $P > 0.05$ ; \*:  $0.05 < P < 0.01$ ; \*\*:  $0.01 < P < 0.001$ ; \*\*\*:  $P < 0.001$ . Note high methionine and tyrosine  $R$  for the terebellid polychaetes *Amphitrite* and *Thelepus*.

Species	Substrate														
	Glucose (500μM)			Aspartic (50μM)			Glutamic (50μM)			Methionine (500μM)			Tyrosine (50μM)		
	$R$	$n$	$P$	$R$	$n$	$P$	$R$	$n$	$P$	$R$	$n$	$P$	$R$	$n$	$P$
<i>Brisaster</i>	< 1.0	10	**	1.7	8	ns	1.6	9	ns	1.7	8	ns	2.8	9	**
<i>Molpadia</i>	1.1	19	ns	3.2	14	***	2.9	14	***	2.3	15	***	5.5	15	***
<i>Parastichopus</i>	1.0	12	ns	1.4	9	ns	1.5	8	ns	1.8	9	**	3.0	6	***
<i>Arenicola</i>	< 1.0	10	*	1.3	9	ns	1.9	9	ns	3.1	10	**	4.8	10	**
<i>Amphitrite</i>	1.4	17	ns	1.6	17	*	1.6	17	ns	11.0	18	***	16.0	16	***
<i>Thelepus</i>	1.1	20	ns	2.6	18	***	3.0	19	***	8.2	17	***	17.0	19	***

**Fig. 1**

Digestive tract of a holothurian.

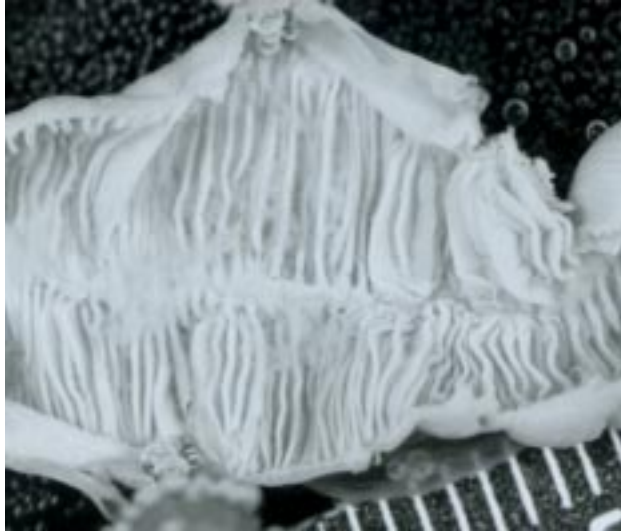


Holothuroidea

(from Rosati, 1968)

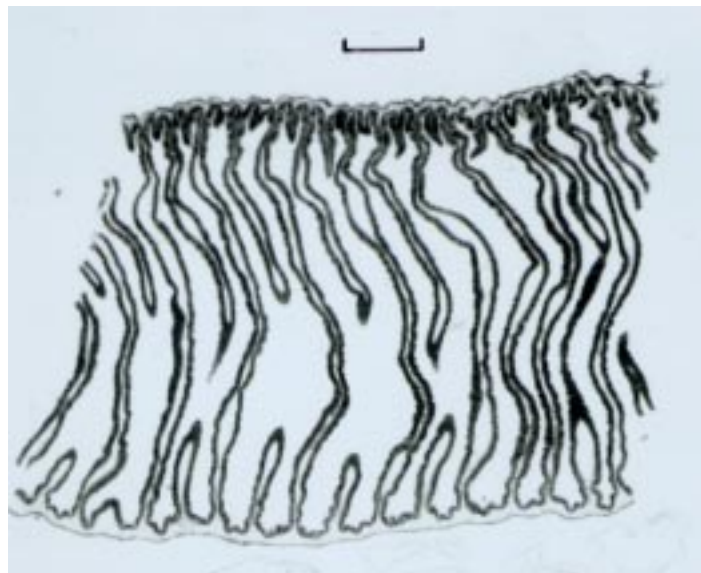
**Fig. 2**

Descending small intestine (foregut) of *Parastichopus* showing transverse lamellae. Ruler spacing equals 1 mm.



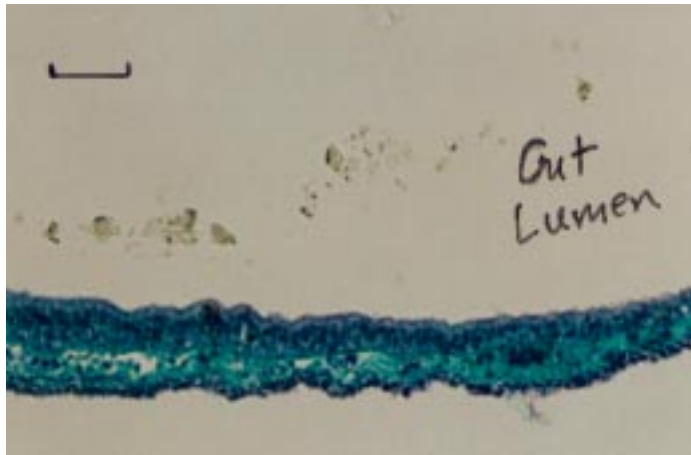
**Fig. 3**

Descending small intestine (foregut) of *Parastichopus*, longitudinal section, showing transverse lamellae. Scale bar = 2 mm.



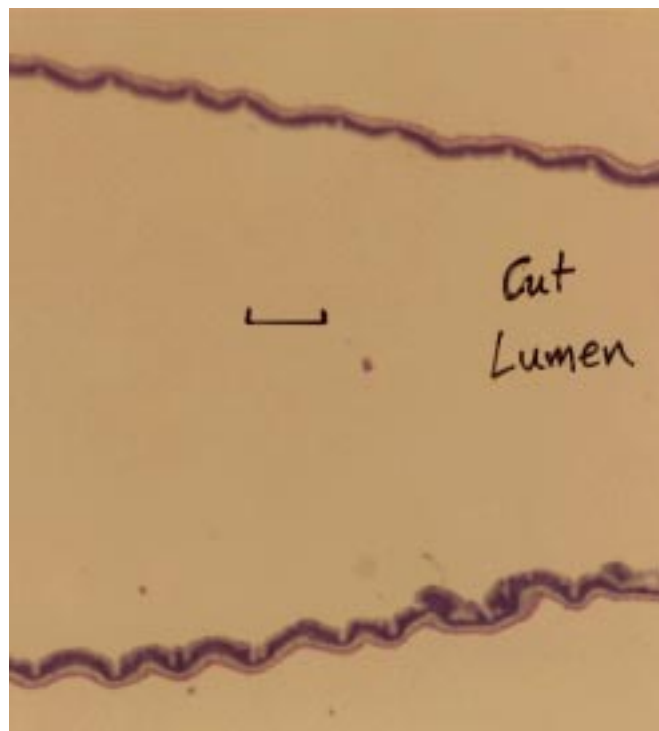
**Fig. 4**

Descending small intestine of *Molpadia*, cross section. Scale bar = 62  $\mu$ m.



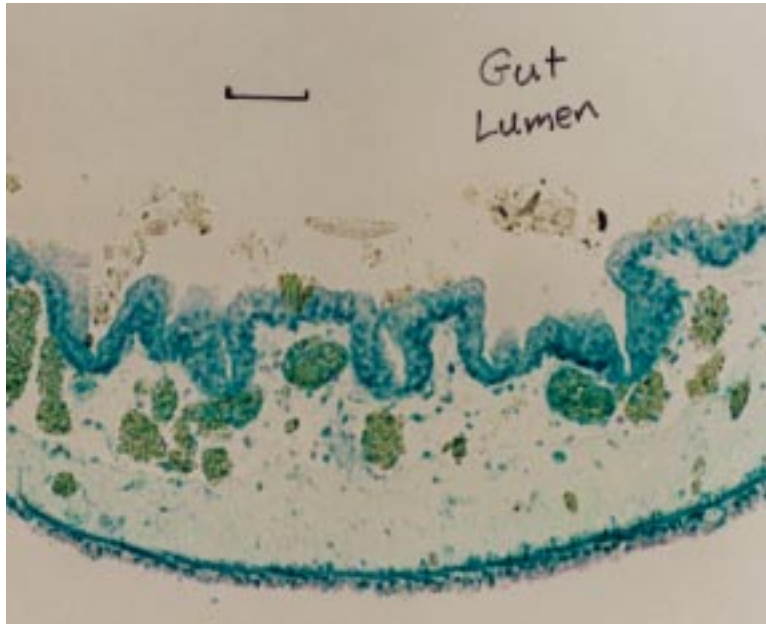
**Fig. 5**

Large intestine of *Parastichopus*, longitudinal section. Scale bar = 1.2 mm.



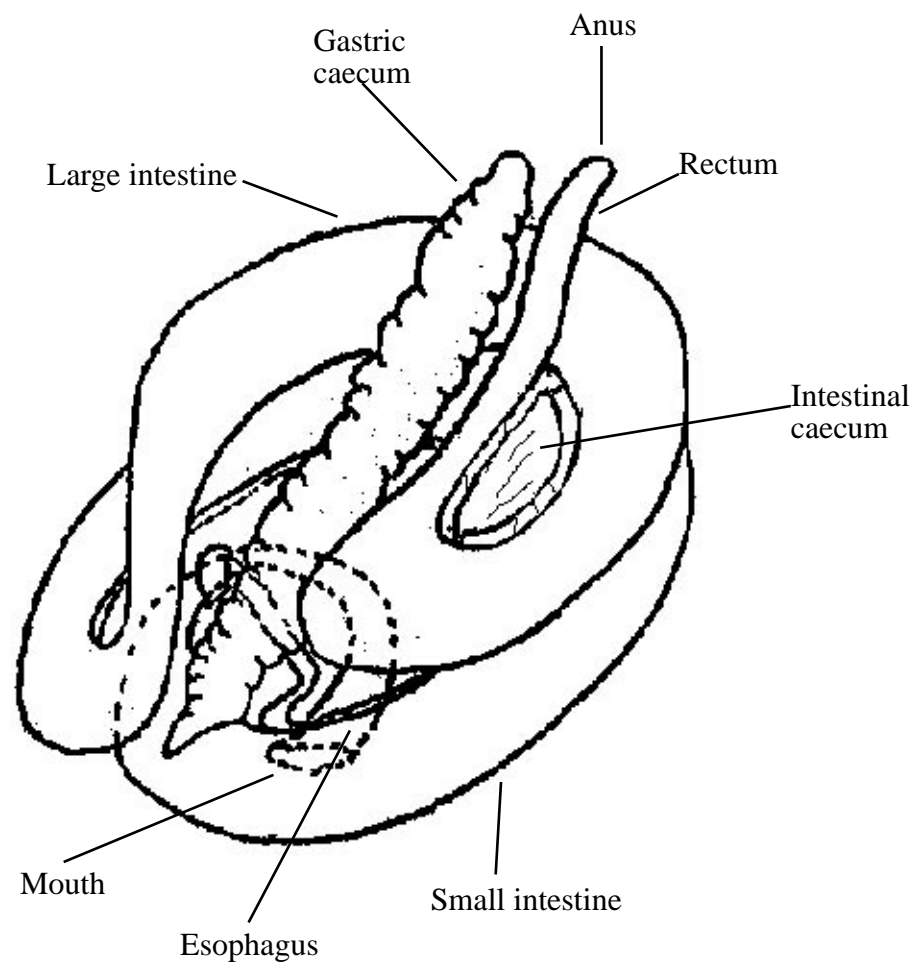
**Fig. 6**

Large intestine of *Molpadia*, cross section. Scale bar = 62  $\mu$ m.



**Fig. 7**

Digestive tract of *Brisaster*.

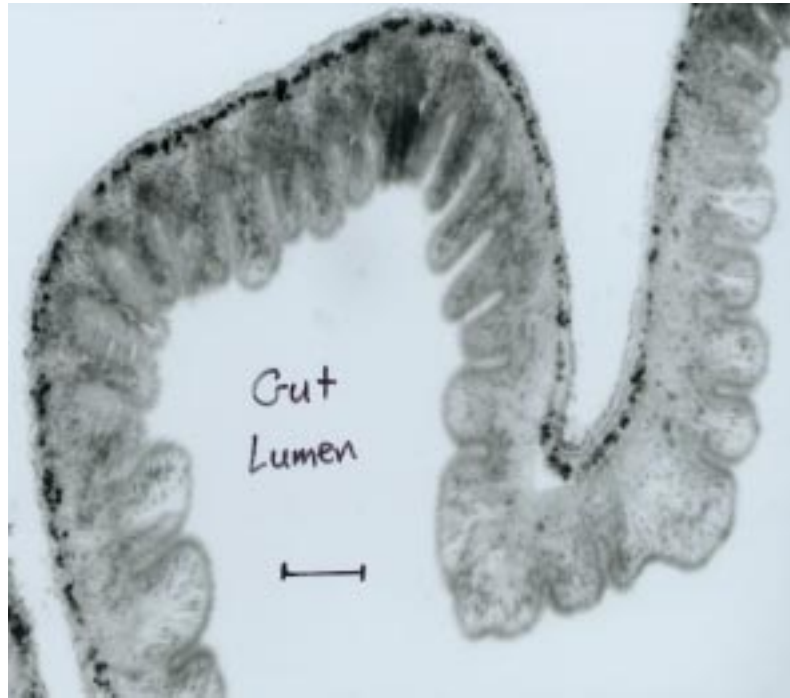


*Brisaster latifrons*

(after DeRidder & Jangoux, 1993)

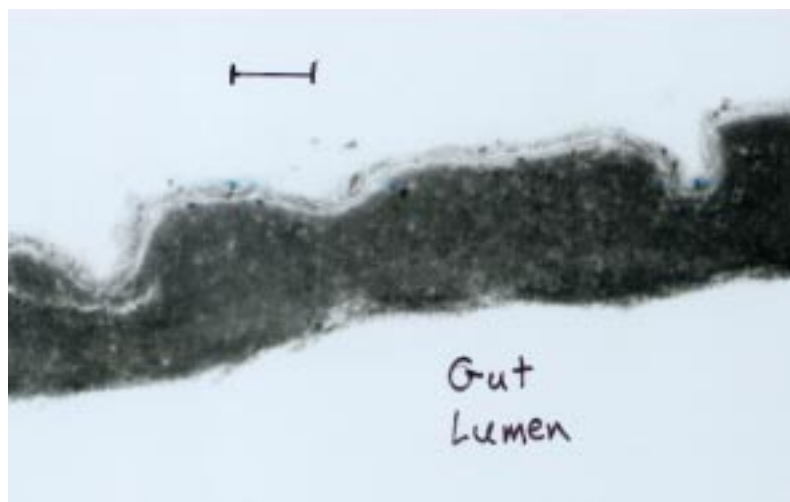
**Fig. 8**

Small intestine of *Brisaster*, longitudinal section, showing transverse folds of gut wall and transverse folds of epithelium within the gut-wall folds. Scale bar = 50  $\mu\text{m}$ .



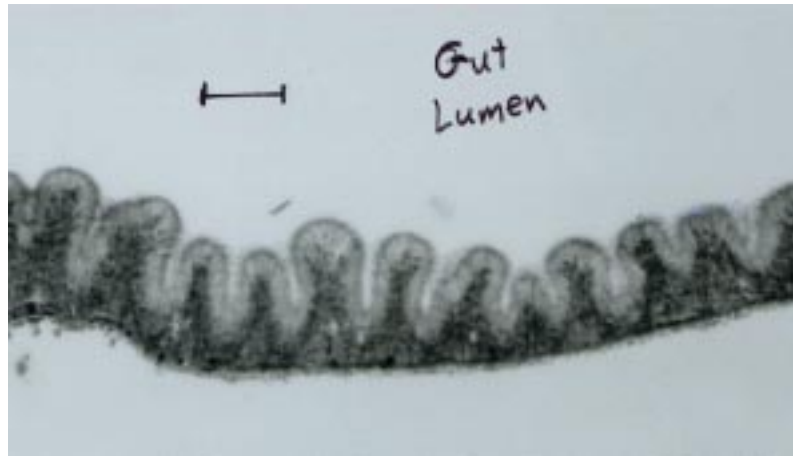
**Fig. 9**

Large intestine of *Brisaster*, longitudinal section. Scale bar = 63  $\mu\text{m}$ .



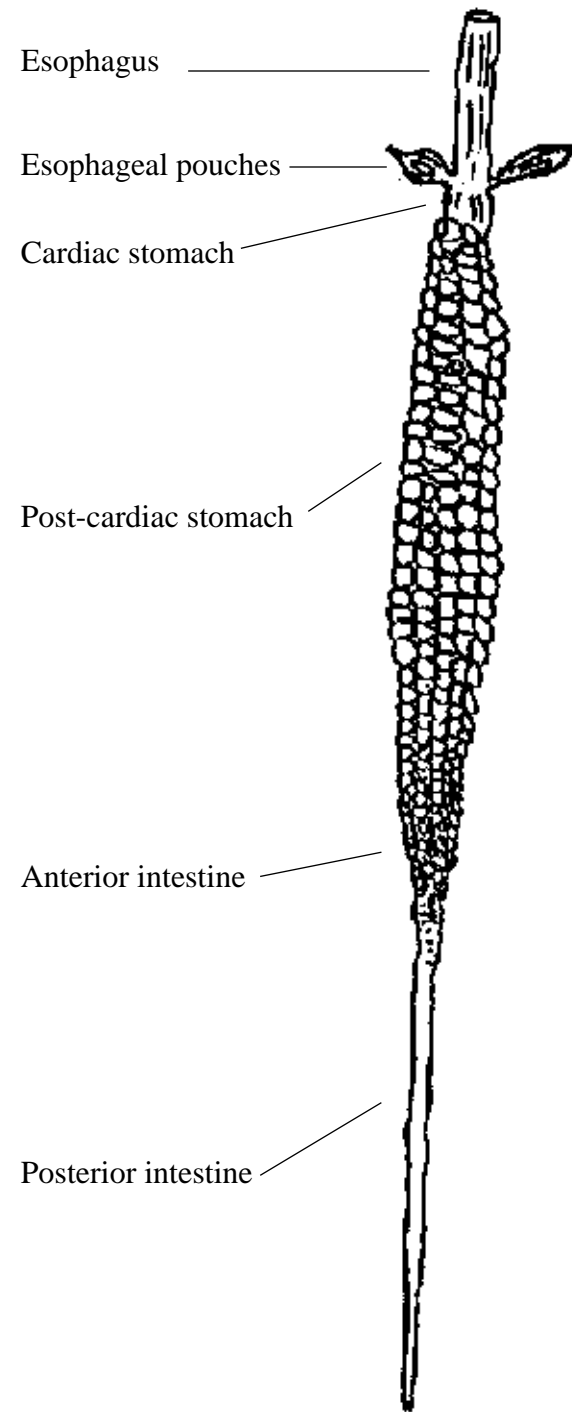
**Fig. 10**

Gastric caecum of *Brisaster*, longitudinal section. Scale bar = 67  $\mu\text{m}$ .



**Fig. 11**

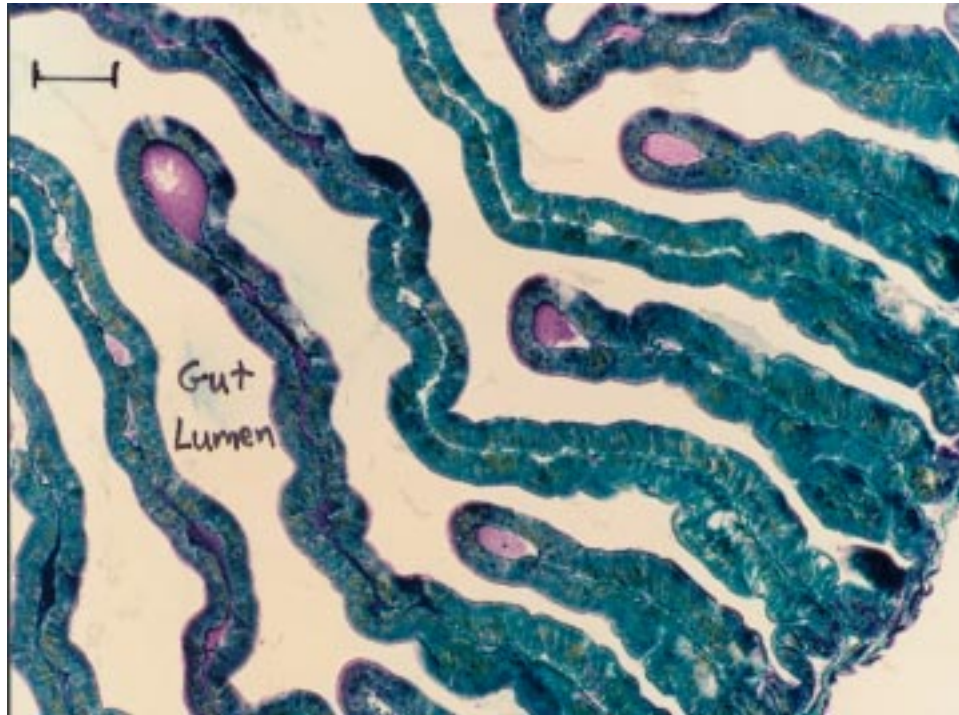
Digestive tract of *Arenicola*.



*Arenicola marina*

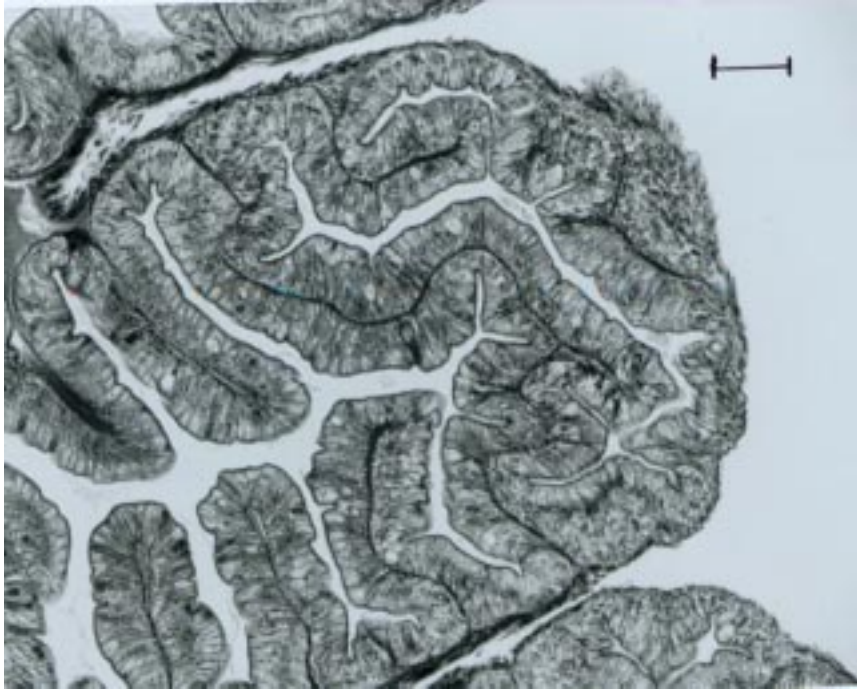
**Fig. 12**

Esophageal caecum of *Arenicola*, cross section, showing narrow folds of epithelium. Scale bar = 62  $\mu\text{m}$ .



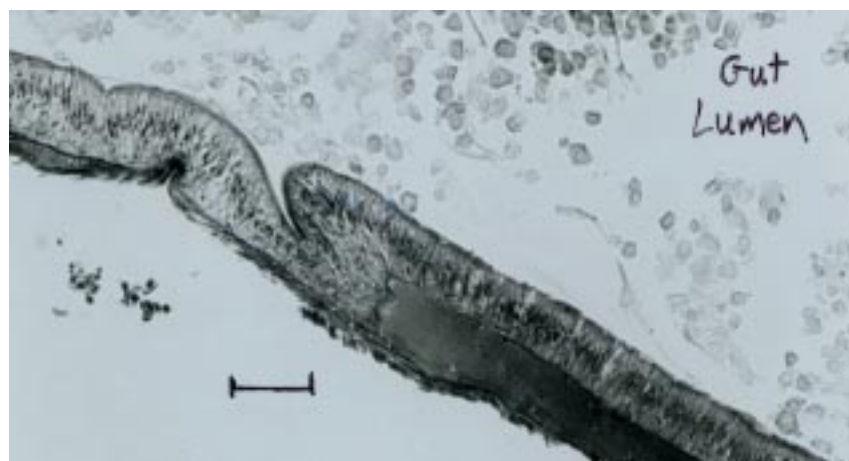
**Fig. 13**

Post-cardiac stomach of *Arenicola*, longitudinal section, showing the highly folded epithelium within a pouch. Scale bar = 62  $\mu\text{m}$ .



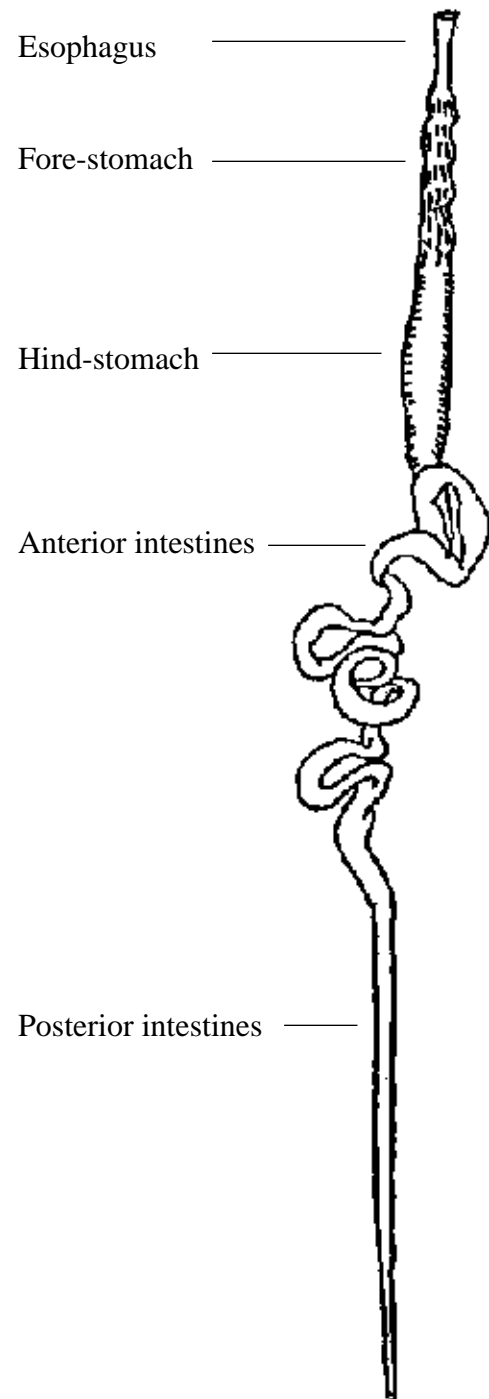
**Fig. 14**

Posterior intestine of *Arenicola*. Scale bar = 62  $\mu\text{m}$ .



**Fig. 15**

Digestive tract of *Amphitrite*.



*Amphitrite johnstoni*