THE DIGESTIVE TRACT OF A HARPACTICOID COPEPOD,

*Tigriopus californicus.*

A LIGHT AND ELECTRON MICROSCOPE STUDY.

By

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Abstract

A study on the digestive tract of a harpacticoid copepod, *Tigriopus californicus*, was carried out using techniques of light and electron microscopy. It was found that a curved, cuticulized esophagus extends from the ventral mouth to the midgut. Its musculature and shape allows fairly large food particles to enter the gut. The noncuticulized portion of the digestive tract consists of: 1. A single, anterior, spherical midgut caecum, 2. An anterior midgut extending from the midgut caecum to the joint at the beginning of the urosome, 3. A posterior midgut extending almost the length of the urosome. The cuticulized hindgut can be divided, structurally, into anterior and posterior regions. It is suggested that the anterior hindgut functions in ion and water regulation as well as begins the formation of a faecal pellet. The posterior hindgut compacts the faecal pellet and retains it until defaecation.

At the light and electron microscope levels four cell types could be distinguished. By studying the cell's position in the gut, electron density, amount of lipid, amount and type of vesiculation and the abundance and position of the cell's organelles, functions for these cells were determined: 1. Cell type one is an embryonic cell which will replace cells worn away or lost in secretion. 2. Cell type two functions mainly in the synthesis and secretion of proteins and also plays a role in lipid absorption. 3. Cell type three appears to function mainly in lipid absorption. 4. Cell type four also functions in lipid absorption but this cell is only found in the anterior midgut.
and the type of vesicles found in this cell suggest a different type of absorption is occurring than in cell type three.

From the abundance of each cell type, the length of the microvilli, the development of the basal lamina and luminal projections, the following conclusions were made: 1. The midgut caecum functions mainly for absorption of digested nutrients. 2. The anterior midgut also functions for nutrient absorption but plays a more important role in merocrine and exocrine secretion. The presence of concretions in cell types two and three of the anterior midgut suggest a role in excretion, water or ion regulation. 3. The posterior midgut functions mainly in absorption, though some holocrine secretion is evident.
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INTRODUCTION

Few studies, at both light and electron microscope levels, have examined the complete digestive tract of any crustacean. This study, on the digestive tract of a harpacticoid copepod Tigriopus californicus, is meant to fill this gap for marine intertidal copepods. Comparisons of the digestive tracts and their cells in other arthropods are made in order to show their differences and similarities. By such comparisons, the functions suggested for the morphology of the gut regions and their cells in other arthropods may be related to T. californicus.

In studies of the arthropod digestive tract confusion often arises over the differences between the terms diverticulum, caecum, digestive gland and hepatopancreas. A diverticulum is generally defined as a blind-ending tubular or sac-like out-pushing from a cavity and thus is a very general term. In this study a caecum was defined as a diverticulum specifically of the digestive tract and is not extensively branched. Digestive diverticula that are extensively branched are called digestive glands or hepatopancreas (Meglitsch 1972).

Studies on other copepods, such as Calanus finmarchicus (Dakin 1908; Marshall and Orr 1955) and Diarthrodes cysteocus (Fahrenbach 1961) suggested five main regions of the gut: a muscular foregut (esophagus) lined by cuticle, three differentiated but not isolated midgut regions including an anterior midgut diverticulum, and a cuticularized hindgut occupying the last abdominal segment(s). Caligoid copepods have
a foregut, two anterior diverticula and a midgut which ends at
the junction between the cephalothorax and the fourth thoracic
leg. Posterior to the midgut is the hindgut (Lewis 1961).

In branchiopods, *Artemia salina* was found to have a
foregut, a midgut with two caeca and a hindgut (Hootman and
Conte 1974). Schultz and Kennedy (1976) described the overall
shape and cells of a Cladoceran (*Daphnia pulex*) digestive tract.

Studies of various malacostracan orders show similarities
in that they all have foregut and hindgut regions. However, the
form of the midgut and the number and form of the midgut caecum
varies. The isopod, *Armadillidium vulgare*, has a midgut in the
form of a hepatopancreas (Vernon, Herold and Witkus 1974) while
amphipods have a true midgut with an excretory caecum extending
from the posterior region of the midgut (Shyamasundari and Rao
1976). *Caridina laevis*, a shrimp studied by Pillai (1960), has
three midgut regions with three blind anterior midgut caeca as
well as a hepatopancreas.

Few studies have been done on the arthropod foregut. Murthy
(1975) has studied the cuticle of the cockroach
(*Periplaneta americana*) foregut and Thomson and Rolling (1976)
have proposed a model to explain the peristaltic and
antiperistaltic activities of the blowfly (*Phormia regina*)
foregut. Knowledge of the phenomenon of antiperistalsis in the
arthropod gut is extremely important to the understanding of
where digestion, secretion and absorption of nutrients occurs
and how they are related to each other. Peristalsis occurs in
the esophagus, anterior midgut and hindgut while antiperistaltic
movements occur in the foregut and posterior midgut. This
allows absorption to occur in the anterior midgut and digestion in the posterior midgut (Vonk 1960).

The permeability of the foregut cuticle is under question. Yonge (1924) stated that no absorption occurs in the esophagus. On the other hand, Eisner (1955) found fat was absorbed in the cockroach foregut and Joshi and Agarwal (1977) found cholesterol was absorbed by the foregut of some omnivorous and carnivorous insects. Mucus and possibly amylase are secreted in this region of the gut (Vonk 1960).

Electron microscope studies on the midgut caecum of various crustacea have suggested functions for this gut region. Ong and Lake (1970), in studying a calanoid copepod described the midgut caecum cells and concluded that they did not produce enzymes but churn and produce mucopolysaccharides as well as absorb amino acids. In various Peracarida it was found absorption occurs in the midgut caecum (Donadey 1969) and Moritz, Storch and Buchheim (1973) suggested: 1. resorption, 2. storage of lipid, 3. storage of glycogen, and 4. secretion occur here. In a eucarid, Callinectes sapidus, glycogen is stored in the "Midgut Glands" (Winget, Rouse and Maurer 1977).

The malacostracan midgut structure and cell components have been studied and may be used to understand the functions of the T. californicus midgut. The midgut by definition is of endodermal origin but Holdich (1973) suggested the isopod midgut is ectodermal in origin and therefore may not be a true midgut. McMurrich (1897) also studied the midgut of terrestrial isopods. Transport of glycine and sodium across the marine shrimp (Penaeus marginatus) midgut was studied by Ahearn (1976) and van
Weel (1955) studied secretion, restitution and resorption in the midgut glands of another decapod (*Atya spinipes*). In the brown shrimp (*Penacus aztecs*) two types of midgut epithelia were observed. There were dark cells with abundant rough endoplasmic reticulum (E.R.), ribosomes and mitochondria with a light matrix and there were light cells (Talbot, Clark and Lawrence 1972).

In malacostracans the hepatopancreas carries on similar functions as the midgut of *T. californicus*. The hepatopancreas, functioning for food absorption, purine metabolism (Vonk 1960), secretion of digestive enzymes and storage of lipids, glycogen and minerals has been studied in malacostracans (Dorman 1928; Davis and Burnett 1964; Hartenstein 1964; Bunt 1968; Loizzi 1968; Stanier, Woodhouse and Griffin 1968; Loizzi and Peterson 1969; Steves 1969; and Lawrence 1976). Schultz (1976), in a study of an amphipod (*Gammarus minus*) described: 1. light staining R-cells which had large numbers of lipid droplets and few golgi bodies; 2. F-cells with many dilated golgi bodies and 3. large vacuolated B-cells with apocrine secretion. These cells were also described by Reddy (1938) in the crab (*Paratelphusa hydrodromus*) and Loizzi (1971) in the crayfish.

Many studies have been carried out on insect midguts and by looking at the cell types described, comparisons can be made between these cells and those in *T. californicus*. Differences between cells and the kinds of cells in different regions of the gut suggest functions for these regions. Sud (1968) described the principal and goblet cells of insects in general. In *Calliphora erythrocephala* (a fly), de Priester (1971) described
columnar (absorptive) cells, regenerative cells and a third cell type which he suggested may be endocrinal in function. The cecropia midgut has "Goblet cells", which are suggested to function for potassium transport from haemolymph to the midgut lumen, and columnar cells but lack regenerative cells (Anderson and Harvey 1966). Flower and Filshie (1976) studied the membranes and junctions of "Goblet cells" in Lepidopteran larvae and Schultz and Jungreis (1977), using scanning electron microscopic techniques, tried to relate the cell's structure to the function of ion transport. Lipophilic and cuprophilic cells were found in the blowfly larvae midgut (Waterhouse and Wright 1960). This copper-accumulating region was studied by Filshie, Poulson and Waterhouse (1971) in Prosophila larvae and by Sohal, Peters and Hall (1977) in the adult housefly. The structure of a mosquito (Culex tarsalis) midgut has been described by Houk (1977) and differences in cells between the anterior and posterior midgut regions of male and female mosquitoes (Rudin and Hecker 1976) and sugar cane beetles (Protaetia acuminata) (Cheung and Low 1975) were studied. In caligoid copepods two cell types were found. A non-vacuolated, absorptive cell of a squamous sort is found in the posterior midgut while a columnar cell is found in the anterior midgut (Lewis 1961). The vacuolated, secretory type cells are squamous after undergoing merocrine secretion (Lewis 1961). A dragonfly (Aeshna cyanea) has specialized smooth E.R. in its midgut cell apices and they are suggested to function in the resynthesis of triglycerides (Andries 1977).

Midgut musculature in Prosophila was studied by Gartner
Mori (1969, 1976) described the formation of the visceral musculature and origin of the midgut epithelium in a Hemiptera (Gerris Paludum insularis). A developmental and cytological study of the fleshfly (Sarcophaga bullata) midgut was undertaken by Nopanitaya and Misch (1974).

The types of secretory processes are explicitly defined by Rhodin (1974) and Kurosumi (1961). Rhodin defines apocrine secretion as secretion where a "droplet lifts the cell surface together with a rim of surrounding cytoplasm". The ruptured membrane is mended and the cell is not lost. Merocrine secretion (a synonym for exocytosis), according to Rhodin (1974), is where the "boundary membrane of the secretory granule fuses with the cell membrane and the contents of the granule are discharged into the lumen". In holocrine secretion cell organelles, including the nucleus, disintegrate changing the entire cell into a huge secretory substance which is extruded in toto (Alikhan 1969). Kurosumi's (1961) definitions of these processes are similar but include subcategories. Apocrine and eccrine (exocytosis) secretion are considered to be under a single light microscopic category of merocrine secretion. Apocrine secretion is divided into macro- and micro-apocrine secretion depending on the quantity of material 'decapitated' from the cell. Eccrine secretion includes processes both visible and non-visible at the electron microscope level. The latter is thought to be a molecular form of secretion through the intact plasma membrane.

In various arthropods, descriptions and functions for the cuticularized hindgut have been given. Lipid, triolein and
disaccharides are absorbed by the cuticularized anal vesicle of a braconid wasp \textit{(Microplitis croceipes)} \cite{Edson1977}. The blowfly \textit{(Calliphora erythrocephala)} rectum was described by Gupta and Berridge \cite{Gupta1966}. They described three cell types: rectal, junctional and cortical cells. The cortical cell is suggested to function in ion and water transport. The rectal pads of the cockroach \textit{(Periplaneta americana)} have also been described and a similar system of solute and non-solute-coupled water transport suggested \cite{Oschman1969}. An Ouabain-sensitive enzyme \cite{Tolman1976} and cellular junctions \cite{Noirot1976} have been implicated in this process.

In crustacea peristaltic movements continually draw water into the gut via the mouth and anti-peristaltic movements continually draw water into the gut via the anus. These processes, their function and means of eliminating the excess water accumulated, have been described by Fox \cite{Fox1952}. Fox \cite{Fox1952} suggested that the functions for such water intake are: 1. acts as an enema for defaecation and 2. stretches gut walls to initiate antiperistaltic muscle contractions. Excess water is passed through the gut wall into the blood and out of the body. Using radioisotopes, Dall \cite{Dall1967} experimented with this system and concluded that water uptake and salt excretion take place in the gut of hypo-osmoregulating crustacea. The hindgut of a terrestrial isopod \textit{(Armadillidium vulgare)} was described \cite{Vernon1974} and confirms an earlier study on another terrestrial isopod \textit{(Oniscus ascellus)} in which microtubule bundles were reported. It was suggested their role
was in orientating osmoregulatory cells of the hindgut (Witkus, Grillo and Smith 1969).

Studies on the enzymes of crustacea have shown the presence of lipases (Eisner 1955; Gilbert and O'Connor 1970), esterases, amylase, maltase (Alikhan 1969) and saccharase as well as protein digesting enzymes such as proteinase, carboxypeptidase, aminopeptidase and peptidase (Bond 1934; Vonk 1960). Microorganisms in the hindgut have been suggested to supply digestive enzymes for cellulose and hemicellulose digestion in the American cockroach (Bignell 1977). In crustacea pH differences are known to occur in different regions of the digestive tract and this affects the activity of various enzymes (e.g. Sinha 1975—invertase activity in a fly).

The purpose of this study was to describe, at the light and electron microscope levels, the digestive tract of a harpacticoid copepod, Tigriopus californicus. From such a description, and comparisons with similar results in other arthropods, it is hoped that functional relationships between the cells and regions of the gut will become evident.

By studying the processes of secretion, digestion and absorption in T. californicus, similar processes in other crustacea will be better understood. By using T. californicus, an intertidal marine copepod, it may be possible to understand and predict the effects disturbances in the marine environment would have on marine food webs.
**MATERIALS AND METHODS**

*Tigriopus californicus* specimens were obtained from a culture maintained by Dr. A. G. Lewis. The species identity was confirmed by Dr. P. L. Illg (U. of Washington). The copepods were maintained in sea water at room temperature in the laboratory under florescent light conditions. Goldfish food and mixed algal cultures were supplied as food and the sea water was changed approximately every three months. Once a good bacterial culture was developed in the culture dishes, continued feeding was no longer necessary.

The material was prepared for light microscope study in the following manner: A 2-hour primary fixation was carried out either in 5% glutaraldehyde or 5% glutaraldehyde and 5% paraformaldehyde in 0.2M phosphate buffer (pH 7.3). After washing in the buffer, the copepods were post-fixed in 2% osmium tetroxide in phosphate buffer for a minimum of five hours, followed by rinsing in buffer and distilled water. The specimens were then stained in 5% uranyl acetate in distilled water. After washing, and appropriate dehydration they were embedded in either Spurr's or J.B. 4 embedding media (Polyscience, Warrington, Pennsylvania).

Specimen blocks were trimmed and 1- to 2-micron sections were cut using a Reichert OMU3 Ultramicrotome or a Du Pont-Sorval JB4 microtome. Sections were floated onto an ethanol-distilled water solution on a cleaned microscope slide. The slide was then heat dried and the sections stained with Toluidine Blue or Aniline Blue Black. Sections were observed
under the Zeiss Photomicroscope.

The difficulty in preparing organisms such as small copepods for electron microscopic study is perhaps responsible for the relative lack of research in this area. As experienced by Rigdon and Mensik (1976) in the brown shrimp, *Penaeus aztecus*, post-mortem degeneration occurs rapidly along the digestive tract. The basic problem in fixing and subsequent processing steps of such tissues is the relative impermeability of the cuticle.

In preparations for electron microscope study, paraformaldehyde was found to reduce post-mortem changes in the tissue, owing, perhaps, to its comparatively fast penetration through the cuticle. In this work 3% paraformaldehyde was used in conjunction with buffered glutaraldehyde (3%) as the primary fixative. Secondary fixation, as in the preparation for light microscope sections, was in 2% osmium tetroxide in phosphate buffer. To ensure dehydration of the tissue a prolonged dehydration in alcohol was used (30-60 minutes exposure for each step of graded alcohol solution) followed by a prolonged bath in propylene oxide. To compensate for the slow penetration of embedding resin through this formidable cuticle barrier, a 7 to 10 day infiltration period was allowed.

The polymerized blocks were trimmed and sectioned on a Reichert OMU3 Ultramicrotome with a Du-Pont diamond knife. The sections were mounted on Colloidion or Formvar coated copper grids and stained, first in a saturated solution of Uranyl Acetate for 30 minutes followed by 10 minutes in lead citrate (Reynolds 1963). The specimens were observed on a Zeiss EM9S
Preparation of material for the scanning electron microscope involved fixing the tissue for one hour in 2% glutaraldehyde in cacodylate buffer. After washing, the copepods were freeze dried and mounted on aluminium stubs by Pelco Colloidal metallic paint.
RESULTS

The scanning electron micrographs of adult male and female *Tigriopus californicus* copepods (Figures 1a and 1b) show the copepod's body shape. A mating pair of *T. californicus* is shown in the light micrograph (Figure 1c). A longitudinal section of the digestive tract of an adult female *T. californicus* is illustrated in its entirety in Figure 2. On a morphological basis the digestive tract shall be subdivided into the esophagus, midgut caecum, anterior midgut, posterior midgut and two regions of the hindgut. The following descriptions are referring to the general adult *T. californicus* copepod.

Esophagus

The cuticular esophagus lies centrally in the anterior quarter of the cephalothorax, approximately 100um from the rostrum. From the mouth at its anterior ventral end, the tubular esophagus curves slightly in an anterior direction. Dahl (1956) also observed this form of esophagus in other copepods. In *T. californicus* the ventral esophagus has two large setae projecting into dips in the esophagus cuticle. The esophagus opens into the midgut at the junction of the anterior midgut and the midgut caecum (Figures 6 and 11). In cross section the lumen of the esophagus frequently takes the form of an "H", depending upon the size and quantity of food that may pass to the midgut via the esophagus (Figure 3). Food in the ventral esophagus lumen is coarser relative to that in the dorsal esophagus lumen. It is apparent that this "H" form reflects the ability of the esophagus to enlarge and constrict.
freely when the need arises.

The lumen of the esophagus is enclosed in three layers. A distinct cuticle separates the esophagus lumen from the nucleated, homogeneously dense, epithelial cells 5-15μm tall. These cells are themselves bound by a circular muscle layer of approximately 10μm in thickness. Longitudinal musculature lies in lateral association with the esophagus.

The cuticle is made up of three layers. The epicuticle is a very thin (0.02μm) layer of electron dense material forming a smooth, unbroken margin between the lumen and the inner cuticle layers. The middle layer is less dense and of uniform thickness (0.2μm) and the innermost cuticle layer, which varies greatly in width, is of low electron density.

The epithelial cells are bound by a plasma membrane which makes deep invaginations, both at the basal regions of the cells as well as at the cuticular regions of the cells. Each cell contains an often irregularly shaped nucleus (0.7μm diameter) with an electron dense circular and central nucleoli (0.2μm diameter). There is little dispersed heterochromatin. Mitochondria are fairly abundant, especially in the region where the esophagus enters the midgut. The electron dense mitochondria are mainly circular or slightly oblong in profile with a diameter of about 0.4μm. The number of cristae, per sectional view, varies but is fairly low. Little endoplasmic reticulum (E.R.) is found but ribosomes and metabolites are abundant throughout the cytoplasm and give it a dense appearance (Figure 16). Frequently, multivesiculate electron dense bodies are evident, especially where the esophagus occurs in the gut.
lumen. Microtubule-like structures (0.02\textmu m diameter) extend in many directions parallel to the esophagus cuticle lining the lumen (Figure 18). Directly apposed to the epithelium is a layer of circular muscle with longitudinal muscle extending between the dorsal and ventral arms of the esophagus. The A, H, I and Z bands of the circular muscle sarcomers (averaging 2.5\textmu m long) resemble those of vertebrate skeletal muscles.

Once the esophagus has reached the junction between the anterior midgut and the midgut caecum, it protrudes into the gut lumen. At this point the esophagus is no longer ensheathed by circular muscle and the epithelial tissue is drawn away from the cuticle (Figure 17). The organelles of the epithelial tissue are essentially the same as before they enter the midgut lumen, except for an increase in unidentified, electron dense, multivesicular bodies. At the point where the esophagus has penetrated the midgut lumen, the cuticle lining the esophagus lumen is continuous with the cuticle lining the outer perimeter of the esophagus. Where the esophagus is continuous with the midgut the cuticle is replaced by microvilli.

Although there were no signs of secretion, some digestion had occurred in the lumen of the esophagus as colloidal material was frequently seen.

**Midgut Caecum**

The midgut caecum is a spherical chamber averaging 40\textmu m in diameter (Figures 4 and 5). It lies anterior to, and above the esophagus at approximately 15\textmu m from the anterior tip of the copepod. The caecum is 20-25\textmu m beneath the dorsal body surface and is located at a constriction forming the junction between
the esophagus and the anterior midgut. The constriction is approximately 60-70um below the dorsal surface of the animal.

The columnar cells of the constricted region are among the largest cells lining the digestive tract and, as throughout the whole midgut, plasma membrane tight junctions (Zonula occludens) occurred at cell apices. The caecum epithelial cells appear to be pseudostratified columnar cells and contain many vesicles at their apical ends (Figure 4). The nuclei are located in the middle or basal regions of the cells. Even at the light microscope level microvilli form a distinct brush border over the entire region (Figures 4 to 6). Deep furrows and spherical cavities suggest secretory activities of large vacuoles along these surfaces.

The epithelial cells of the caecum are columnar with an average height of 7.6um (Figure 19). At the base of the cells there are numerous interdigitations of the plasma membrane (Figure 21). Although these together with the basal lamina may extend into the cytoplasm up to one third of the height of the cell they more commonly penetrate only 0.6um into the cytoplasm of the cell. These basal lamina invaginations can, occasionally, be seen to pinch off the basal region of a cell. In these regions the basal lamina forms a concentric ring around the basal region of the cell almost isolating it from the rest of the cell and midgut caecum. It is in these irregular invaginations that longitudinal muscle is most obvious. No circular muscle was seen to surround this region of the midgut although the basal lamina may appear, in some regions, to be a form of muscle. It is most evident (averaging 0.2um wide) in
regions where only minor invaginations occur.

The mitochondria are fairly scattered throughout the cells with a possible increase in numbers at the apex and base. They are rather small (0.6um diameter) and circular in profile and possess a variable number of lamellate cristae (two to many with a predominance of 8 cristae per mitochondrial section) which extend the length of the organelle. The mitochondrial matrix is homogeneously dense so that cristal membranes may become obscured.

Ribosomes are often associated with E. R. The latter is extensively developed in most cells and, along with free polysomes, tends to aggregate basally and along nuclear and plasma membranes. The large circular or slightly convoluted nuclei lie centrally or basally and average 3um in diameter. They contain scattered areas of heterochromatin and/or distinct nucleoli (1um diameter). The pars amorpha and nucleonema of the nucleolus are frequently evident at higher magnifications.

The microvilli in this region of the midgut are 2um long (Figure 19). They form a comparatively compact row lacking a definite peritrophic membrane while having a 'membrane' resembling a glyocalyx lying parallel and between the microvilli. Occasionally there are regions where the microvilli have been pushed aside and small gaps occur. The microvilli, in cross section, appear to contain a minimum of 6 microfibrils which can also be seen extending the length of the microvilli when cut longitudinally. A longitudinal section also shows an electron dense region at the tip of the microvilli although no terminal web at the apex of the cell could be observed.
At the ultrastructural level differences between cell types are even more striking than at the light microscope level. Cell type one is seen to lie at the base of the gut epithelium in clusters of three or more cells (Figure 12 and 23). In a cross section of the midgut caecum an average of three of these clusters is evident. Basal laminal indentations often involved the cells adjacent to this cell type but seldom actually penetrated the cytoplasm of cell type one. The central nucleus, with its single or occasionally double nucleolus, is only slightly larger than the average nucleus of this region. Some mitochondria, rough E. R., and golgi are randomly distributed in the cytoplasm of these cells. The cytoplasmic density of the cells seems to be mainly a result of loose ribosomes. Some vesicles, averaging 0.5μm diameter, are present in these cells. Most of these vesicles, though membrane bound, were electron transparent. Occasionally a cell contained vesicles with an electron dense core. These vesicles were 0.2μm in diameter. Lipid inclusions were not observed in these cells and the cells were never observed to reach the lumen.

In the midgut caecum, approximately one cell in seven is cell type two (Figures 19 and 24). This cell type has regular invaginations of the basal lamina and has a nucleolated central nucleus. The randomly dispersed mitochondria are slightly more abundant than in cell type one. Rough E. R. and loose ribosomes make the cytoplasm appear extremely dense. The golgi bodies are usually so dilated that individual cisternae can seldom be discerned. These golgi are abundant throughout the cytoplasm and are intimately associated with vesicles (averaging
0.07 μm diameter) which contain a core of dense material and can be seen to fuse with each other. When the vesicles become larger (0.3μm diameter) the density of their contents becomes greatly reduced until eventually they appear as large (1.0μm) electron opaque vesicles. These vesicles have been seen to undergo exocytosis (Figure 20). From the sections taken, it appears this cell type is pyramidal in its three dimensional shape. The cells are usually wider at the base with a pointed apex or wider at the apex with a smaller base. In all cells of this type there was a portion of the cell reaching the lumen and having microvilli. Small lipid vesicles were occasionally seen at the very apex of the cell.

In addition to exocytosis, mentioned above, holocrine or merocrine secretion was observed at the light microscope and electron microscope level (Figures 14 and 22). Cytoplasmic masses of various sizes containing identifiable membrane bound organelles were frequently seen in the lumen of the digestive tract. Through serial sections these masses could be traced to cell type two. After this secretion process, the density of these cells was greatly reduced.

Cell type three, the most prevalent cell type, has the ultrastructure of a typical exocrine cell (Figures 19 and 24). The basement membrane is invaginated, the nucleus is generally basal and is surrounded by varying amounts of lamellate rough E. R. Electron micrographs of this cell type show more portions of randomly distributed mitochondria than in the other two cell types. Golgi bodies are typically located in association with the nucleus and vesicles of all sizes (0.2-2.0μm) are seen in
the apical half of the cell. Some of the larger vesicles may contain evenly distributed electron translucent or opaque particulate material. Fusion between vesicles is a common occurrence. Many fairly small vesicles at the apex can be seen fusing to the plasma membrane in the regions between microvilli (Figure 20). Lipid droplets were also seen at the apex of these cells.

**Anterior Midgut**

The anterior midgut extends approximately 350μm beyond the junction of the esophagus with the midgut caecum and ends at the second constriction of the digestive tract at the position of the third pair of swimming legs (pereiopods). This region of the gut lies consistently 50-70μm below the dorsal surface and 40-50μm from the ventral surface of the copepod body. Its lumen averages 65μm in height but increases in cross sectional width from 60 to 150μm towards the posterior end (Figure 5).

The pseudostratified cuboidal cells of the extremely convoluted anterior midgut average 8μm in height and have a basal lamina, as described for the caecum, which may extend 0.6μm into the cytoplasm of the epithelial cell bases (Figure 25). In the anterior midgut the number of semi-circular evaginations from the basal regions of the cells has greatly increased when compared to the midgut caecum. As in the caecum each evagination has longitudinal muscle associated with it. Circular muscle is more prevalent in the anterior midgut but is still not continuous.

Each cell has a relatively central oblong nucleus (2.5μm by 1.5μm) containing a central nucleolus and/or scattered
heterochromatin. Mitochondria in these cells appear to be evenly distributed throughout the cells. They are fairly small (0.2um diameter) and circular or slightly elongated in shape and contain well developed parallel cristae. The three cell types of the midgut caecum are present, though less extreme in cytoplasmic density differences, and there is possible evidence for a fourth cell type (Figures 25, 26 and 29).

Cell type one is just as prevalent in the anterior midgut as in the midgut caecum and maintains the same ultrastructure (Figure 28). Cell type two appears to have even more rough E. R. in this region of the midgut than the caecum (Figure 26). Most of this rough E. R. is in the basal two-thirds of the cell, being so compact in the basal half of the cell as to virtually exclude all other organelles. At the cell apex the cisternal rough E. R., typical of the base, has become less continuous and less well defined. The golgi bodies of this cell type, as in the caecum, are abundantly distributed throughout the cell and are not necessarily associated with the nucleus. The large golgi bodies are composed of many dilated cisternae completely surrounded by small (0.04um diameter) vesicles. Most of the golgi-associated vesicles are slightly electron opaque or transparent. These vesicles become larger (0.4um diameter) and are often seen to have membrane-like structures as inclusions. The occasional golgi body is associated with vesicles which are more electron dense and have a very electron dense central core. These vesicles were described in the midgut caecum and, as in the caecum, they become larger and the core becomes less electron dense as they progress to the cell apex. Another type
of vesicle not observed in the caecum is fairly electron dense and contains a cluster of very electron dense bodies. These bodies sometimes appear to be composed of, or surrounded by, membranes. Holocrine or merocrine secretion of the apical third of these cells occurs in this region of the gut as well as in the caecum (Figure 25). These cells have numerous lipid-like, homogeneously electron opaque, droplets 0.1-0.3um in diameter.

Cell type three has a much smaller supply of rough E. R. which is more evenly distributed throughout the cell (Figures 26 and 28). Its broken and dispersed nature, as well as the presence of circular cross sections of rough E. R., suggest that at least some of the rough E. R. is tubular. Though still dilated, the golgi bodies are noticeably smaller and fewer in number than in cell type two of this region of the midgut. The electron transparent vesicles of these cells are of comparable size and number as in cell type two and also contain loose membrane structures as inclusions. Vesicles with electron dense cores, characteristic of cell type two, were essentially absent from these cells. Lipid droplets were observed at the cell apices.

The cytoplasm of the fourth cell type, with its basal nucleus, is similar in electron density to cell type three but differs from this cell type in a variety of ways (Figures 25, 26 and 29). Rough E. R. in these cells is even more broken up and rare. The golgi bodies of these cells are smaller and slightly less dilated than in cell type three. Electron transparent vesicles of similar size and number as in cell type three occur and are evenly distributed however, cell type four is
characterized by another type of vesicle. This cell type has an extremely abundant supply of small vesicles (0.1-0.4um diameter) containing material of various degrees of electron density. These vesicles dominate the apical third to half of the cell. Cell type four extends from the basal lamina to the lumen but appear to lack microvilli.

The microvilli in this region are shorter than in the midgut caecum (1um) (Figure 28) and occasionally lie flat in areas where food or adjacent cells are apposed. Neither a glycocalyx nor a peritrophic membrane were evident in this region of the midgut although small, apparently hollow vesicles (0.07um diameter) were randomly dispersed at the apices of the microvilli.

When food material was seen in this region of the gut it was already partially digested. This was indicated by the lack of recognizable organelles inside the food cells. Instead, one finds diatom frustules that are still together but only contain material slightly greater in electron density than that distributed throughout the gut lumen. Occasionally bacteria cells were seen, either as independent cells or in clusters of three to eight cells.

**Posterior Midgut**

The posterior midgut extends approximately 175um from the anterior midgut to the hindgut. The lumen height is fairly constant at 45-60um with a width of 100-110um (Figure 7). This region of the midgut lies in the center of the body cavity surrounded by approximately 20um of body tissue.

The unfurrowed low cuboidal or pseudostratified squamous
cells of this region average 3-4um in height (Figure 15). They appear, under the light microscope, to be much more uniform in structure and staining properties than the cells of the anterior midgut and, in contrast to that region, the cells of the posterior midgut contain no or few secretory vesicles (Figures 30 and 31). The brush border of these cells appears thinner (0.4-0.6um) than those of the anterior midgut.

The epithelial cells of the posterior midgut have a less convoluted basal lamina than more anterior regions of the midgut. The basal lamina extends 0.5um into the cytoplasm. Circular muscle (0.4um wide) and longitudinal muscle is much more common, though still not consistently present, in this region of the midgut. Extremely flattened nuclei (40um long by 0.7um tall) are located in the widest portion of the cells and so, owing to the pseudostratified nature of the tissue, there may be a second nucleus located between a nucleus and the midgut lumen. The nuclei either contain one dense circular or slightly oblong nucleolus (1um by 0.6um) but more commonly contain a fairly scarce supply of diffuse heterochromatin.

The three cell types originally found in the midgut caecum are present in the posterior midgut. Cell type one is more common than in the more anterior regions of the midgut and is present next to the basal lamina (Figures 32 and 33). These cells are especially elongate and flattened. They have mitochondria (0.7um long by 0.3um diameter) with lamellate cristae extending the length of the mitochondria. The matrix of the mitochondria is electron dense. Rough E. R. is scarce though the cells are filled with electron dense particulate
material. Golgi bodies were not observed.

Cell type two is even more evident in this region of the midgut than in the anterior midgut (Figure 31). Its pyramidal shape, with a narrow base and wider apex, is greatly exaggerated. In some regions this cell type is separated by only 3-4 cells of type three and the arms of the apex of this cell type may extend to touch the next arm of its neighbouring cell type two (Figures 30 and 31). A central or apical nucleus is found in these cells and has a similar electron density as the rest of the cell matrix. A central nucleolus is common. Some electron dense mitochondria and rough E. R. could be observed but the main characteristics of the cell are its extreme electron density and its vesicles. The vesicles (0.2um diameter) appear as less electron dense regions in the cell. In the center of these vesicles there is usually a more electron dense circular region. These vesicles accumulate at the apex of the cells and there is evidence that they may play a part in merocrine secretion. The holocrine-type of secretion is less common in the posterior midgut than in the more anterior regions of the midgut. Holocrine secretion, however, was observed (Figures 32 and 33). Occasionally a larger electron opaque vesicle (1.8um by 0.7um) occurs in the apical region of the cell.

The microvilli of this cell type (averaging 0.5um long) are not as compactly arranged as in other regions of the midgut. They are frequently associated with large masses of medium electron density, particulate material. These masses of particulate material were about 17um deep. They followed the
gut epithelium the length of the apical arms of cell type two. The variation in electron density of this material was not as great as in the anterior midgut and a membrane, possibly the peritrophic membrane, was more consistently present around this mass of digested material. Food material, in the form of whole bacterial cells and empty diatom frustules was immersed in this material.

The third cell type is less electron dense than cell type two and has a regular basal lamina (Figures 30 and 31). Its nucleus tends to be slightly more irregular in its outline and contains dispersed heterochromatin and/or a spherical nucleolus. The mitochondria (1μm long and 0.3μm diameter) have a slightly less electron dense matrix than cell type two mitochondria but the number and shape of the cristae appear the same. The plasma membrane of this cell type frequently forms interdigitating folds with rough E. R. following the plasma membrane of both cells involved. The rough E. R. is fairly scarce and takes the form of short sections loosely following the plasma membrane. Golgi bodies are abundant and are made up of 5-7 undilated cisternae. Electron transparent, membrane bound vesicles bud off from these cisternae. These cells extend from the basal lamina to the gut lumen.

**Hindgut**

The total length of the hindgut is 80-90μm but it appears to be subdivided into two regions (Figure 9). Closest to the posterior midgut and centrally located is a region 30-40μm in diameter and length. This portion of the hindgut is lined by elongated wedge-shaped epithelial cells with the lumen
characterized by numerous narrow clefts and lined by a layer of lightly stained cuticle (Figures 8 and 35). From this region to the dorsal anal opening, located between the uropods, is the second portion of the hindgut. This region is frequently dilated and is heavily cuticularized. This posterior region of the hindgut lacks or has a minimal epithelial lining (Figure 36) and the lumen is often filled with faecal pellets.

**Anterior Hindgut**

The simple squamous epithelial cells of the hindgut average 3μm in height (Figure 8). They have a basal lamina similar to that seen throughout the digestive tract except that the invaginations are much reduced and less complicated. The nuclei of these cells are fairly circular (1.5μm diameter) and contain the regular scatterings of heterochromatin. Any E. R. and the few golgi that are found in these cells occur around the nuclei. Mitochondria in these cells are predominately elongate in cross section (about 1μm long) and contain well developed cristae in a dense matrix. Microvilli, so characteristic of the midgut, are gradually replaced by cuticle similar to that described for the esophagus (Figures 34 and 35). This is the region of the digestive tract where defined faecal pellets may be found, surrounded by a peritrophic membrane.

**Posterior Hindgut**

Between the anterior hindgut and the anus is the last region of the copepod digestive tract (Figure 14). Here the nuclei, lying in the center of the simple squamous cells, are elongate (2.5 μm long) and lie with their main axis parallel to the cuticle (Figure 36). The mitochondria are evenly
distributed and are slightly elongate (0.5 μm long) with few cristae in a matrix of low electron density. E.R., golgi and vesicles of any description are essentially absent.

The posterior hindgut is lined by cuticle similar to that found in the esophagus and anterior hindgut except that the middle region of the cuticle (the endocuticle) is thicker (0.7 μm thick) and takes on the striated appearance typical of the cuticle protecting the outside of the copepod (Figure 37). Within or just inside of the epicuticle (outermost dense region of the cuticle) electron transparent circles are evident (0.03 μm diameter) (Figure 38).
Examination of the shape of the digestive tract of *Tigriopus californicus* reveals that it is a typical copepod digestive tract. As in calanoid copepods (Lowe 1935) and harpacticoid copepods (Fahrenbach 1961), the digestive tract of *T. californicus* is made up of a cuticularized esophagus, a midgut divided into two regions with a single conical anterior extension, and a cuticularized hindgut. There is a fair degree of regional structural specialization which can, in general, be correlated to particular functions of the gut regions. The noncuticularized regions of the digestive tract, however, also showed continuities in basic structure and cell characteristics.

**Esophagus**

The characteristics of the *T. californicus* esophagus are similar to those described for the crayfish (Yonge 1924), calanoid copepods (Dakin 1908; Lowe 1935; Marshall and Orr 1972), caligoid copepods (Lewis 1961), and another harpacticoid copepod (Fahrenbach 1961) and even a cladoceran (Schultz and Kennedy 1976). The muscle system seen in this copepod esophagus and extensively described for other harpacticoid copepods (Lang 1948; Fahrenbach 1961), creates a structural base for the shape of the esophagus and undoubtedly functions in controlling the extent of dilation and relaxation of the esophagus lumen. These dilations and relaxations may serve to control the speed of the movement of food to the midgut. The 'H' shape of the esophagus lumen indicates a high degree of contractility and would allow great variability in the amount of material that can be ingested.
In the ventral portion of the esophagus fairly large setae were observed passing through the esophagus lumen. These setae, like those of the cockroach (Murthy 1975), do not appear to act as a filtering mechanism. However, they may function in mastication. The setae exist in association with a dip in the cuticle lining and thus, along with the arms of the ventral esophagus, form a type of mortar and pestle. The fact that the particulate material in the ventral arm of the esophagus is much coarser than that found in the more anterior and dorsal portions of the esophagus suggests a certain degree of physical breakdown.

There is a question as to whether the microtubules seen throughout the esophagus epithelium and especially in direct association with the cuticle may be involved in enzyme and/or mucus export by the esophagus cells. It is most likely that these microtubules take on the more structural function of maintaining the esophagus shape. The microtubules are probably not involved in the export of enzymes and/or mucus from the epithelial cells because there is little sign that the epithelial cells are secretory. Although the mitochondria of these cells are small, they are relatively abundant to supply the energy required for the esophagus and its cells to undergo conformational changes. Although little synthesis of enzymes occurs in the esophagus there are indications that digestion has taken place. This same situation was seen in a millipede and so it was suggested that digestive enzymes were passed into the esophagus from the midgut (Nunez and Crawford 1976).

The dense multivesicular bodies found in the epithelial
cells increase in number as the esophagus approaches the midgut. This may suggest, along with the apparent decrease in cuticle thickness, that some material is being absorbed and possibly accumulated in the esophagus. This may also be a region where minerals and ions are regulated. Evidence that the cuticle is not an impenetrable barrier and could function in such processes is seen in the fact that in omnivorous and carnivorous insects, cholesterol is absorbed in the foregut (Joshi and Agarwal 1977). Yonge (1924) stated that there was no absorption in the esophagus although the cuticle was semipermeable. Bisner (1955) found that incompletely digested fat is absorbed in the cockroach foregut.

In the midgut of the housefly, *Musca domestica*, concretions containing phosphorous, chlorine, calcium, iron and copper occur and are similar in appearance to the electron dense structures found in the *T. californicus* esophagus (Sohal, Peters and Hall 1977). In the housefly, these concretions were thought to play a role in excretion. These concretions were also reported in another harpacticoid studied by Fahrenbach (1961). In the shrimp, salt (NaCl) excretion occurs in the anterior diverticulum (Dall 1967).

Musculature

The musculature of the region from the caecum to the posterior midgut shows a trend. There is a fairly irregular arrangement of striated circular muscle and longitudinal muscle in the caecum. The muscles of the anterior midgut becomes a fairly continuous arrangement of circular muscle by the posterior midgut. The most evident longitudinal muscle, as in
Calanus (Lowe 1935), occur in association with the midgut evaginations. From serial sections of Tigriopus californicus, it appears that these evaginations may in fact be spherical bodies attached to the main portion of the midgut by a narrower neck portion. This observation makes it less conclusive that this set of muscles is, in fact, longitudinal. Instead, they could be circular, surrounding or pinching the neck portion of these evaginations. The function for such a muscle system is not clear but it is obvious that a longitudinal muscle system in conjunction with the circular muscles would be useful in peristaltic type contractions.

It is known that peristaltic and antiperistaltic contractions of the gut occur in the blowfly (Thomson and Holling 1976), Daphnia (Schultz and Kennedy 1976) and calanoid copepods (Marshall and Orr 1972). In Daphnia peristalsis moves food through the esophagus to the anterior midgut and antiperistalsis moves digestive juices from the posterior midgut to the anterior midgut. This system is suggested to be a mechanism for digestion in the anterior midgut, secretion in the posterior midgut and movement of nondigestible material out of the digestive tract as faeces (Vonk 1960; Schultz and Kennedy 1976). In the cockroach, lipase is known to be secreted by the midgut and the midgut caecum, yet it is found in the foregut. Eisner (1955) suggested antiperistaltic contractions in the foregut may facilitate this movement of enzymes. This system of food flow is reminiscent of the digestive and filtering system of malacostracans (Meglitsh 1972).

In decapods, amphipods and calanoid copeods the non-
striated longitudinal myofilaments lie between the circular myofilaments and the basal lamina (Dakin 1908; Lowe 1935; Loizzi 1971; Schultz 1976), while in isopods the longitudinal filaments are external to the circular (Donadey 1971). Schultz and Kennedy (1976) suggested the muscle system of Daphnia is helical and lacks the true circular or longitudinal muscle characteristics. From the appearance of the caecum and anterior midgut 'longitudinal' muscle that is not associated with the evaginations, the muscle system of *T. californicus* appears to be similar to that of Daphnia. In these regions the 'longitudinal' muscle appears more as if it were circular muscle cut in a diagonal fashion. The muscle of the posterior midgut appears to be much less longitudinal or circular and may be helical. If in fact the muscle system of this copepod is a continuum, it would be concluded that at the posterior midgut the helix has become so tight that it is lined entirely by muscle which, when sectioned, always reveals a diagonal or oblique view. In fact, in the region of the posterior midgut the muscle is no longer striated. As in the crayfish (Yonge 1924), the hindgut lacks circular muscle. This is probably associated with a lack of regular peristalsis. Fahrenbach (1961) found the hindgut undergoes a series of twitches during defecation rather than smooth peristalsis.

Loizzi (1971) described the myo-epithelial network of the crayfish hepatopancreas as being composed of longitudinal muscle fibers formed from branches of circular fibers such that the resulting perpendicular myofibrils shared the same sarcolemma. Yonge (1924) proposed that the function of the hepatopancreatic
muscle system was to make possible the movement of digestive juices out of the hepatopancreatic tubule and of partially digested nutrients into this tubule. He concluded that contraction of the circular muscle produced the outward flow while contraction of the longitudinal fibers caused the inward flow. If the muscle system is as described by Loizzi (1971), then contraction would involve both longitudinal and circular muscles simultaneously so that inefficient ballooning would not occur. If the midgut caecum and the anterior midgut systems are similar functionally to the hepatopancreas, then it seems reasonable to expect the same type of musculature as in the hepatopancreas.

The muscle system in *T. californicus*, as described in this study, would allow similar peristaltic, antiperistaltic and pumping actions of the gut as discussed in other arthropods above. With additional information concerning the amount of digestion that has occurred in the various regions of the *T. californicus* gut and observations of where absorption has occurred, it seems most probable that the same situation occurs as in *Daphnia*. That is, antiperistaltic contractions move food and digestive enzymes from the posterior midgut to the anterior midgut and midgut caecum where absorption occurs.

**Basal Lamina**

The basal lamina of the midgut appears to follow an opposite trend to that of the musculature of the midgut. In the midgut caecum and anterior midgut the basal lamina is most prominent and shows its greatest invaginations and complexities. In the posterior midgut the basal lamina tends to form delicate
dips at random intervals. The function of the basal lamina has been suggested to be water (Pease 1956) and ion regulation (Filshie, Poulson and Waterhouse 1971; Hootman and Conte 1974; Burgos and Gutierrez 1976). The classic function of any type of surface area expansion is to increase surface area for exchange. The extensiveness of the basal lamina suggests that it functions in exchange of material of some sort between the gut epithelium and the underlying haemocoel (Beams and Anderson 1957). It would follow, therefore, that the greatest amount of exchange between the underlying tissues and the gut epithelium occurs in the caecum and the anterior midgut.

In the rat, Na, K-ATPase activity has been shown to be high in the basal lateral intestinal epithelium (Crane 1975; Mircheff and Wright 1976). The structure of the basal lamina is that of a grid in association with an amorphous material and this association is suggested to serve as a mechanical support and an avenue for filtration (Terzakis 1967). The fact that mitochondria of the epithelia are often found in close association with these invaginations suggests an active metabolic role (Waterhouse and Wright 1960; Nopanitaya and Misch 1974). The mitochondria would supply the energy required for transport of materials against a concentration gradient.

Cell types one, two and three were evident to some degree along the whole extent of the midgut and so it seems most appropriate to discuss each cell type as an individual entity and then discuss any variances in number, characteristics or form that may occur in the various regions of the gut.
Cell Type One

The basal position of cell type one in *Tigriopus californicus* and the characteristics of its organelles suggest it to be an undifferentiated cell. These cells would develop and replace cells that had worn away or were lost in holocrine secretion. The distribution of the cells, often in the evaginations almost excluded from the gut epithelium, suggest they are, at this stage of development, independent from the absorptive-secretive function of the gut. If the function of the basal lamina is as discussed above, it would be logical to find that a cell not actively functioning in exchange would have a comparatively smooth basal lamina. The basal lamina subtending cell type one is relatively smooth.

In insects regenerative cells occur in clusters called nidi and it has been suggested that once basal infoldings occur, the cells have already begun differentiation (Sud 1968; de Priester 1971). Although these regenerative cells usually occurred in clusters there was little sign of cell division. In the adult isopod studied by Hartenstein (1964), no mitotic activity was found in the digestive tract.

In *Tigriopus californicus* the abundance of ribosomes with little E.R. or golgi bodies in cell type one suggests that, while protein synthesis is occurring, protein products are not necessarily being exported outside of the cell. Mitochondria are fairly abundant to provide the energy required for the processes of cell division and differentiation. The simple organization of the cytoplasm with few membranes or residual vacuoles but large numbers of free ribosomes is typical of
undifferentiated cells (Smith 1968).

This first cell type of *Tigriopus californicus* appears to be comparable to the E-cells of decapods, amphipods and isopods (Loizzi 1971; Clifford and Witkus 1971; Schultz 1976). These E-cells are embryonic cells forming the distal mitotic region of the hepatopancreatic epithelium. This embryonic region of the hepatopancreas undergoes cell division to produce an intermediate cell type (T-cell) which then differentiates to a light cell type (L-cell). In the L-cell, secretory products begin to form (van Weel 1955) and accumulate until the L-cell becomes an extrusion cell at which time merocrine secretion occurs (Pillai 1960). The empty cell is now the dark cell (D-cell) which is able to restore itself to the light cell again and continue through this cycle of secretion. In this situation the embryonic cells of the hepatopancreas replace cells that are worn away.

Although *T. californicus* does not have a hepatopancreas, the E-cell of this organ in other Crustacea can be compared to the undifferentiated embryonic cell of *T. californicus*.

**Cell Type Two**

The second cell type found in the midgut of *Tigriopus californicus* is seen to be very darkly stained when viewed under the light and the electron microscope. It has abundant rough E. R., ribosomes, mitochondria and dilated golgi bodies with secretory vesicles in all stages of maturation, size and position. All of these organelles and their abundance makes it likely that such cells are synthetic cells. Dilated golgi bodies with loose arrangements of lacunae are suggestive of very
active golgi bodies (Fawcett 1969). Exocytosis was frequently seen in these cells irrespective of their state of maturation. Exocytosis and a process similar to merocrine or holocrine secretion occurred in the mature cells. After this latter process of secretion the cell itself and the secreted products in the gut lumen appear much lower in electron density. The cell loses its microvilli and its organelles become less identifiable but the material being extruded remains intact, for a time.

The shape of this second cell type is that of a wedge, with its base either at the basal or apical end. This shape suggests a process of maturation and differentiation. The cell condition with the broad base probably represents a younger cell, while those possessing the wider apex could be the mature state before merocrine or holocrine secretion. The next step of this process is for the apex of the cell to protrude into the gut lumen, and for the plasma membrane to break down to extrude the cell products and organelles.

Another fairly common characteristic of this second cell type is the accumulation of lipid droplets in the apical third of the cell. These droplets are homogeneously dense and have a fairly consistent diameter. This, along with the occurrence of endocytosis, suggests that absorption of digested products was occurring in these cells.

The secretory stage of this cell type may be considered its ultimate function. From the organelles and their abundance it is obvious that this cell functions in protein synthesis. While the cell is synthesizing it appears to also function in lipid
absorption. The basal lamina invaginations of these cells adds credence to the idea that these cells are directly active in the metabolism of the gut. This is in contrast to crabs (Reddy 1938) and caligoid copepods (Lewis 1961) where the dark cells are apparently only involved in secretion.

This cell type most approaches the F-cell (Fibrillar cell) of decapods and amphipods (Loizzi 1971; Schultz 1976). The F-cell is described as being fibrillar with a basophilic cytoplasm, developing vacuoles and functioning in enzyme synthesis. In crabs these cells are called 'dark cells' (van Weel 1955; Pillai 1960). These cells are described as having a faintly frothy cytoplasm with minute fibrils extending from the base of the cell to its apex (Pillai 1960). In the isopod hepatopancreas the basophilic cells are B-cells (Clifford and Witkus 1971).

In copepods, randomly spaced columnar cells with a very densely staining cytoplasm have been described. These cells are not as frequent as other cells in the gut. In caligoid copepods they have been called type B cells and their granules have been suggested to function in extracellular digestion (Lewis 1961). In calanoid copepods this 'dark' or 'compact' cell type is also present (Lowe 1935; Marshall and Orr 1972) and they are described by Dakin (1908) as being smaller, more regular, hexagonal cells that are very wide for their depth. Fahrenbach (1961) did not describe a cell comparable to cell type two.

Cell type two of the T. californicus digestive tract, therefore, is similar to the enzyme synthesizing cells of other crustacea in both structure and function.
Cell Type Three

Cell type three is common throughout the noncuticularized gut of *T. californicus*. It is much lighter in staining characteristics than cell type two and tends to have larger, less electron dense secretory vesicles. The intermediate amounts of rough E. R., golgi, mitochondria and loose ribosomes suggest secretion is not a major function of this cell. The function of this cell may be indicated by the small vesicles of lipid material and smooth E. R. that accumulate in the apical portions of the cell. Thus, it appears that absorption is their major function and synthesis is limited to intracellular metabolism of the cell products absorbed.

These cells show a greater and more consistent development of basal infolding than in cell type two and it could be suggested that this third cell type is active in transport between the gut lumen and the haemocoel.

In insects there does not appear to be a differentiation between the cell types two and three so the cells that are not regenerative or goblet cells are called 'principal cells' by Sud (1968) and 'columnar' cells by Smith (1968). These cells are said to function for both absorption and secretion. Smith (1968) suggested that although some pinocytosis may occur, most nutrients are taken up directly through the apical plasma membrane as small molecules not detectable in the electron microscope.

Decapods and amphipods have two cell types that appear to fit the function and description of the third cell type of *T. californicus*. The first is a storage cell (R-cell) which
also functions in absorption and contains nutrient stores (Loizzi 1971; Schultz 1976). The second is a blister-like cell (B-cell) which secretes digestive enzymes and contains a single large vacuole and a scanty basophilic cytoplasm (Loizzi 1971; Schultz 1976). The isopod hepatopancreas has acidophilic cells (S-cells) which may be comparable to the third cell type under discussion for T. californicus (Clifford and Witkus 1971). The 'light cells' of the crab digestive tract are columnar, narrow cells that tend to vacuolize (Pillai 1960). According to van Weel (1955), these cells are most common in the midgut gland of shrimp (Atyidae) and are a stage of a cell cycle in which secretory products are formed and stored before being extruded. In calanoid copepods these 'light' cells are large and their heavy vacuolation forces the cytoplasm and nucleus to the base of the cells. They also have protrusions extending into the lumen of the gut (Dakin 1908; Lowe 1935; Marshall and Orr 1972).

The function of cell type A in the midgut of caligoid copepods, a cell comparable ultrastructurally to cell type three in T. californicus, is absorption of digested food materials (Lewis 1961). The large vacuolated cells of calanoid copepods were also found in a harpacticoid copepod, Diarthodes cystoeicus, studied by Fahrenbach (1961). The shape of these cells is apparently dependent upon physical factors such as the presence or absence of food in the gut.

Cell type three of T. californicus is comparable in structure to cells in decapods, amphipods and other copepods. The predominating function of these cells varies from order to order. In T. californicus, as in caligoid copepods, cell type
three functions mainly for absorption but undergoes some secretion. In decapods and amphipods, separate cell types carry out each of these functions rather exclusively.

Each region of the noncuticularized *T. californicus* gut had each of these three cell types and a fourth cell type was found in the anterior midgut. Each region of the gut will be discussed independently, pointing out variances and other unique features and their functions.

**Midgut Caecum**

The midgut caecum of *Tigriopus californicus* contains the largest cells of the whole midgut and there are a moderate number of evaginations at the base of the cells. The basal lamina is deeply invaginated. The three cell types described above are present with cell type three being the most abundant, cell type two next followed by cell type one. This fact, along with the relative scarcity of luminal evaginations, suggests merocrine or holocrine secretion is fairly uncommon. A glycocalyx around the microvilli may be present although its appearance is closer to that of the particulate material in the lumen of the caecum or the anterior midgut.

The fact that the microvilli in this region are the longest of the midgut suggests a great absorptive function for this region. Crane (1975) has proposed a membrane model for the active passage of glucose and passive passage of fructose from the gut lumen through the digestive-absorptive microvilli border into the epithelial cells. In the fly anterior midgut the microvilli were the longest of the midgut and were definitely surrounded by a glycocalyx (de Priester 1971). It has recently
been suggested that the microfilaments found inside the microvilli may give it contractile abilities (Rodewald, Newman and Karnovsky 1976). This process would then act to 'pump' material, which has moved inside the microvilli, into the cytoplasm below (Boyd and Parsons 1969).

In *T. californicus*, lipid droplets were very common in the anterior midgut cell types two and three and so it is suggested, as for *Daphnia* (Schultz and Kennedy 1976) and isopods (Donadey 1969), that this is one of the major sites of nutrient uptake. Ong and Lake (1970) believe the midgut diverticulum of *Calanus helgolandicus* does not produce enzymes but churns and produces mucopolysaccharides and absorbs amino acids to be transported by the basal mitochondria pump to the haemocoel.

In malacostracans the midgut caecum stores glycogen (Winget, Rouse and Maurer 1977) and produces most of the digestive enzymes. In *Daphnia* the anterior midgut, as well as the midgut caecum, produces digestive enzymes (Schultz and Kennedy 1976). Pillai (1960) suggests there is very little difference between the cells of the midgut caecum and the cells of the midgut in the shrimp (*Caridina laevis*). Amphipods have storage (R-cells), fibrillar (F-cells) and vacuolated (B-cells) cells in their midgut diverticulum. The function of these cells is resorption, storage of lipid and glycogen as well as secretion (Moritz, Storch and Buchheim 1973). Thus, as in *T. californicus*, both secretion and absorption take place in this region of the midgut.

In calanoid copepods the whole anterior midgut, including the diverticulum, is lined by a uniform layer of nonvacuolated
cubical cells (Lowe 1935). Marshall and Orr (1972) suggest that in calanoid copepods food is constantly pushed backwards and forwards in the wide midgut caecum and anterior midgut. In caligoid copepods, secretory cell type B is primarily in the foregut and anterior midgut regions suggesting this is the region where secretion occurs (Lewis 1961). In the harpacticoid copepod studied by Fahrenbach (1961), the midgut diverticulum is made up of large vacuolated cells projecting into the lumen.

In most of the Crustacea described above, as in *T. californicus*, the midgut caecum or diverticulum functioned mainly in absorption of nutrients. In some crustaceans, the nutrients absorbed are stored and in others the caecum also produces enzymes.

**Anterior Midgut**

In *Tigriopus californicus* the anterior midgut has a basal lamina with the greatest number of evaginations and invaginations. The cells and microvilli are smaller than those of the caecum and the microvilli lack a surface coat. In *Daphnia* (Schultz and Kennedy 1976), the glycocalyx of these microvilli is also not evident. A microvilli glycocalyx is present in the midgut cells of decapods (Talbot, Clark and Lawrence 1972) and the R-cells of the crayfish hepatopancreas (Loizzi 1971). It is lacking in the bilobed hepatopancreas of isopods and amphipods (Clifford and Witkus 1971; Schultz 1976) as well as in the caecum and midgut of *Calanus* (Ong and Lake 1970). This region of the midgut has the greatest number of luminal extrusions and along with deep invaginations from the lumen into the cells, the surface area for absorption and
transport is greatly increased.

Of the three cell types, cell type one appears to be slightly more abundant here than in the midgut caecum and merocrine or holocrine secretion of cell type two is obvious. Cell type three is noticeably more electron dense than in the caecum. The increase in abundance of cell type one suggests a greater loss of gut cells in this region either through wear or through secretion. The density of cell type three may reflect a greater amount of nutrient absorption in this region of the gut when compared to the caecum and, indeed, there appears to be a significant increase in the number of lipid droplets in both cell types two and three.

In this region of the Tigriopus californicus midgut there are two characteristics not found in any other region of the midgut. These are: 1. the presence of cell type four, and 2. cellular concretions in cell types two and three. The vesicles of cell type four, from their shape, size and electron density, are suggestive of either lipid absorption, endocrine secretion or zymogenic secretion. The scarcity of rough E. R. virtually eliminates the last possibility. The possibility of endocrine secretion by these cells is open due to the fact that in insects there is some evidence that the secretion of digestive enzymes is under hormonal control (Wigglesworth 1965). However, for protease production in the insect intestine a cephalic endocrine system rather than an intestinal endocrine system is in control (Garcia and Garcia 1977). The polarity in this cell type four of E. R. and golgi bodies basal and vesicles apical makes it unlikely that the small vesicles in
these cells are endocrine in *T. californicus*. Thus, it appears the most likely possibility for the function of the fourth cell type is lipid absorption.

It is known that lipid absorption may occur by at least two processes. One process would be molecular incorporation by the cell through the plasma membrane such as appears to occur in the cell types two and three. Such a system would occur if digestion were extracellular, as Bond (1934) suggested. In rats sucrase is concentrated at the brush border plasma membrane (Micheff and Wright 1976) and this enzyme is suggested to release glucose from intraluminal sucrase. Therefore the more easily transportable hexose is available for transport through the plasma membrane. In the corn borer, *Diatraea grandiosella*, the enzyme for the substrate is located in the membrane of the cell that will absorb the products of the reaction and is not secreted into the gut lumen (Turunen and Chippendale 1977). Thus, digestion of the lipid is not truly extracellular. In the crayfish hepatopancreas, lipase distribution on the striated border could be correlated with the presence or absence of a filamentous (glycocalyx) layer associated with the microvilli (Loizzi and Peterson 1969). Gilbert and O'Connor (1970) suggested lipases occur extracellularly in arthropods. Lipolysis greatly increased absorption but the fact that it is not a prerequisite for absorption (Weintraub and Tietz 1973) suggests a second means of lipid uptake by cells.

The other process of lipid absorption is pinocytotic or endocytotic incorporation into cytoplasmic vesicles. The vesicles are then passed directly to the E. R. and golgi system
for metabolism (Lentz 1971). Phagocytosis of fat droplets was never observed in the crab and so it was suggested that glycerol and fatty acid products of extracellular digestion were resynthesized into fats in the light cells (van Weel 1955). Although it was not determined which method of lipid absorption occurs in *Tigriopus californicus*, cell types two and three as well as cell type four contain lipid.

The electron dense multivesiculate bodies found in this region of the *Tigriopus californicus* midgut may be concretions formed by an accumulation or excretory process. These concretions may also be residual lysosome bodies as are found in the fly midgut (de Priester 1971). Pillai (1960) found concretions of the shrimp, *Caridina laevis*, to be formed by the midgut cells and believes that they represent secretory products. The gut, with its close association to the external environment and the haemocoel, would be an obvious place for secretory and/or osmoregulatory processes to occur.

Fahrenbach (1962) presumed the anterior midgut concretions in the harpacticoid copepod, *Diarthrodes cystoecus*, to be excretory as in the housefly. The housefly concretions, containing high concentrations of phosphorus, sulfur, chlorine, calcium, iron and copper are initially deposited within golgi vesicles, lamellar bodies and residual bodies (Sohal, Peters and Hall 1977). The fact that the multivesiculate bodies of *Tigriopus californicus* appear to be lamellar or residual bodies suggests they may be concretions of a similar nature to those of the housefly and *D. cystoecus*.

In many insects midgut goblet cells, originally thought to
function as a reservoir of secretion (Sud 1968), function in ion regulation. It was found in the Cecropia midgut that large amounts of calcium and magnesium and lesser amounts of potassium and sodium are taken up by isolated mitochondria. This process was stoichiometrically linked to phosphate uptake and electron transport. In contrast to plasma membrane transport systems which are ouabain-sensitive, neither mitochondrial ion accumulation nor potassium transport across the midgut is inhibited by ouabain (Anderson and Harvey 1966). The overall transport is that of potassium from the haemolymph into the gut lumen to be excreted (Flower and Filshie 1976). The mitochondria, intimately involved with the cell cavity projections of the goblet cells, are thought to be the site of ion exchange. Specialized cell junctions occur to join these goblet cells to the columnar cells. These junctions may control the opening and closing of the goblet cell apex and thus the extrusion of the cavity matrices (Schultz and Jungreis 1977).

Cells similar to these were not seen in Tigriopus californicus but cell types two and three appear to possess a similar function as suggested by the presence of concretions, invaginated basal lamina and mitochondria.

The functions of the insect midgut include; secretion of digestive enzymes, absorption of the products of digestion, absorption and regulation of water (Berridge and Oschman 1972; Cheung and Low 1975), and possibly a storage depot for food reserves (Smith 1968). In the dragonfly it is suggested that resynthesis of triglycerides occurs in specialized midgut E. R. (Andries 1977). In the brown shrimp, Penaeus aztecus, the
anterior midgut cells are hypothesized to function in lipid storage, secretion, peritrophic membrane formation, absorption and possibly osmoregulation (Talbot, Clark and Lawrence 1972).

In the mosquito (Rudin and Hecker 1976) and in Daphnia (Schultz and Kennedy 1976), the anterior midgut is the important organ in absorption since the caecum is relatively small compared to the organisms with large hepatopancreatic organs. In the brachiopod, Artemia, the apical and basal cell amplifications and associated mitochondria are correlated with absorption and osmoregulation (Hootman and Conte 1974). In an early description of the terrestrial isopod midgut, McMurrich (1897) stated that the anterior midgut was not at all glandular or absorptive and that it was lined by impermeable cuticle. He suggested all digestion and absorption occurred in the hepatopancreas.

In calanoid copepods secretion is said to occur in the anterior midgut regions. Often enzyme activity is affected by pH of the medium in which it is present. The fact that there is a definite change in pH in the middle versus the anterior and posterior midgut in Drosophila (Filshie, Poulson and Waterhouse 1971) suggests that the midgut functions in some specific way to suit the gut matrix to the enzymes that it is secreting. The most suitable pH for invertase activity in Diptera occurred only in the most anterior portion of the midgut (Sinha 1975).

Thus, as for insects, shrimp, harpacticoid and calanoid copepods, the anterior midgut of T. californicus functions for both absorption of nutrients and secretion of enzymes. Definite conclusions concerning whether osmoregulation, excretion or
storage of nutrients occurred in the *T. californicus* anterior midgut could not be made although the possibilities exist. In the shrimp *Caridina laevis*, the peritrophic membrane is suggested to be formed in this region of the midgut but it certainly was not obvious in *T. californicus*.

**Posterior Midgut**

The posterior midgut cells of *Tigriopus californicus* are one-half the size of the anterior midgut cells and one third the size of the midgut caecum cells. This, however, may be due to the pressure of food aggregates that are retained in this region of the gut. In the cockroach, the rate of food passage in the gut decreases towards the anus and so it is concluded that digestion occurs in the posterior regions as well as more anteriorly (Bignell 1977). In the corn borer lipolytic enzymes were only secreted by the posterior midgut (Turunen and Chippendale 1977). Proteases accumulate in the posterior midgut of a beetle, *Attenogenus megatoma*, although it is not known whether secretion of the enzyme is actually greater or that the enzymes simply accumulate here (Baker 1976). That an enzyme accumulates in the posterior midgut also suggests that some digestion occurs here.

Lipid absorption occurs in the *Tigriopus californicus* posterior midgut and cell type two, which is suggested to produce enzymes and be involved in secretion, also occurs here. The invaginations and evaginations of this posterior region of the midgut are minimal and thus suggest there is less exchange between these cells and the haemocoel. Although the microvilli are one-half the length of the rest of the midgut and are quite
irregular, absorption still appears to occur to a considerable degree as indicated by the number and size of lipid droplets in the cells. In the fly, poor fixation of the posterior midgut is blamed on the high lipid content of these cells (de Priester 1971).

Only cell types one, two and three are present in the posterior midgut of *Tigriopus californicus* and there are slightly more of cell type one than in the anterior midgut. This suggests that there is a greater need for replacement cells which follows with the finding that secretion of cell type two is more definitely of a holocrine than a merocrine nature. This secretion was also observed in *Daphnia* where gaps in the digestive tract resulted from the total extrusion of groups of three to four cells (Schultz and Kennedy 1976).

In calanoid copepods the cells of the posterior midgut either revert back to being similar to those of the midgut caecum (Lowe 1935) or the whole midgut is made up of similar cells (Marshall and Orr 1972). In caligoid copepods the fact that cell type A predominates in the posterior portion of the midgut suggests that this region is the main site of absorption (Lewis 1961).

In many arthropods the processes of digestion (via proteases, lipases and carbohydrate splitting enzymes (Barns and Goodfellow 1968)), purine metabolism, absorption and storage of glycogen, fat and copper, occur in the hepatopancreas (Vonk 1960). *T. californicus* does not have such an organ but similar cells and the same processes that occur in the hepatopancreas also appear to occur in the *T. californicus* midgut. This
similarity in the cells and function of the *T. californicus* midgut and the hepatopancreas makes it appropriate to briefly discuss the hepatopancreas.

The hepatopancreas of the crayfish is the most thoroughly studied and consists of two identical lobes lying along the gastro-intestinal tract in the dorsal part of the cephalothorax. As in the midgut, exchange between the cells and the haemocoel occurs through the basement membrane of the cells (Dorman 1928). The lobes of the hepatopancreas consist of tubules with embryonic cells distally next to absorptive cells. Secretary and fibrillar cells are more proximally located. From labelling studies it was found that all the cells in the tubules arise from embryonic cells at the apex (Davis and Burnett 1964). Thus, the embryonic cells are at first absorptive, then secretory, then 'fibrillar' (containing abundant E. R.) and finally they die. Loizzi (1968) suggests that the absorptive and basiophilic cells originate separately in the distal tip of the hepatopancreas and only the latter give rise to the secretory cells. In crustacea a major portion of the hepatopancreas was found to be lipid (Steves 1969; Lawrence 1976).

The absorptive cells of the hepatopancreas are termed R-cells and they are comparable to cell type three of *T. californicus*. The R-cells have lipid droplets, short cisternae of rough E. R., smooth E. R. and golgi bodies with flattened cisternae (Loizzi 1971). These cells are occasionally seen to have electron dense inclusions in their mitochondria suggestive of heavy metals (Bunt 1968). In crabs the R-cells
absorb iron and fat (Stanier, Woodhouse and Griffin 1968). According to Schultz (1976) the R-cells absorb copper while the F-cells absorb iron. Cell type two of *T. californicus* is comparable to the fibrillar cells of the crayfish hepatopancreas which are suggested to function in the secretion of a non-zymogenic protein (Bunt 1968). These F-cells also absorb material from the lumen by means of bulk transfer as opposed to contact digestion and molecular transport suggested for R-cells (Loizzi 1971). In isopods the hepatopancreatic epithelium is formed from interspersed small, acidophilic absorptive S-cells and larger, basiophilic absorptive and secretive B-cells with a distal embryonic region which gives rise to both of the latter cell types (Schultz 1976).

The function of the *T. californicus* posterior midgut, like the hepatopancreas, is secretion of digestive enzymes and absorption of nutrients. The major of these two functions in *T. californicus* is secretion while the major function of the posterior midgut of caligoid copepods is absorption.

**Peritrophic Membrane**

The peritrophic membrane, typical of crustaceans, was not consistently evident in *T. californicus* until faecal pellets were formed. Occasionally material was seen in apposition to the microvilli but this was more likely the membrane glycocalyx, material that had been extruded from the cells, or digested material in the lumen.

In isopods this membrane is hypothesized to be formed by the endodermal midgut caecum cells (Holdich 1973). In insects the peritrophic membrane is found around the food in the midgut.
and is reported to be formed either along the length of the midgut or at the narrow cleft between the midgut and the foregut (Smith 1968; Nunez and Crawford 1976). Specialized protein producing cells in the most anterior portion of the midgut produce the first component of the peritrophic membrane (Smith 1968; Filshie, Poulson and Waterhouse 1971; Nopanitaya and Misch 1974). A second layer is supplied by the foregut and a third layer is a thin sheet making a 20μm dense layer containing fibrous material (Smith 1968).

In calanoid copepods the peritrophic membrane, which surrounds the faecal pellets, is secreted by the posterior midgut (Gauld 1975). This membrane is the endodermal equivalent to the ectodermal cuticle and is made up of chitinous (Clarke, Temple and Vincent 1977) microfibrils embedded in a protein and polysaccharide group substance (Schultz and Kennedy 1976).

In the shrimp the peritrophic membrane is an extremely thin and transparent chitinous membrane found in the midgut surrounding the faecal pellets (Forster 1953; Pillai 1960). This peritrophic membrane has been suggested to protect the midgut cells from damage and to act as a fine filtration device (Forster 1953; Smith 1968). Gauld (1975) suggested that the calanoid peritrophic membrane acts to keep the faeces in a compact pellet which will sink rapidly out of the water column in which the copepods are feeding. In isopods an acid mucopolysaccharide coat replaces the peritrophic membrane and is suggested to function in protecting the gut cells (Hartenstein 1964). Burgos and Gutierrez (1976) concluded that the peritrophic membrane is the glycocalyx associated with the
microvilli and is a filamentous material with compartments adjacent to the microvilli. They suggested that this structure could function as a substrate for hydrolytic enzymes. Beams and Anderson (1957) argued that the porous nature of the peritrophic membrane results from the penetrating microvilli. In the brown shrimp the peritrophic membrane resembles the microvillar surface coat and is present, though variable, throughout the midgut (Talbot, Clark and Lawrence 1972). Studies on the brine shrimp, *Artemia salina*, show that fusion of the glycocalyx and/or vesicles between microvilli produce the peritrophic membrane of the midgut and hindgut (Hootman and Conte 1974).

The place where the peritrophic membrane is formed appears to be variable. In calanoid copepods, and possibly in *T. californicus*, it appears to be formed in the posterior midgut. In Cirripedia and Caridea the membrane lines the whole midgut. The peritrophic membrane of *Daphnia* is formed by the foregut. The functions of this membrane (filtration barrier, protection for midgut cells, compacter of faecal pellets) are fairly obvious and acceptable. Food was not consistently present in the *T. californicus* gut nor was the peritrophic membrane. This fact made it difficult to make conclusions on the formation or form of the membrane in *T. californicus*.

**Secretion**

The types of secretory processes that appear to occur in the arthropod digestive tract cover a wide range. Exocytosis of secretory products is evident in all of the noncuticulized portions of the *T. californicus* digestive tract and most probably merocrine or possibly holocrine secretion occurs in the
anterior midgut and midgut caecum. In the anterior midgut the
cells that are extruding into the lumen still have many
organelles and a nucleus. This situation, by definition, is
merocrine secretion. On the other hand, cells are frequently
found in the posterior midgut that have been completely expelled
from the gut epithelium. In this case holocrine secretion
appears to have occurred.

In some decapods secretion in the hepatopancreas has been
stated to be holocrine while in the crab, secretion appears to
be merocrine (van Weel 1955; Stanier, Woodhouse and Griffin
1968). This conclusion was drawn from the fact that there was
not an increase in mitotic figures in the embryonic zone with
increased secretory activity. Secretion in the shrimp (Caridina
laevis) digestive diverticula is merocrine (Pillai 1960).
Digestive enzyme secretion in Daphnia is holocrine (Schultz and
Kennedy 1976) in contrast to the apocrine or merocrine method
suggested by Loizzi (1971) to occur in other decapods, isopods
(Clifford and Witkus 1971) and amphipods (Schultz 1976). In
caligoid copepods secretion of cell type B is merocrine for this
cell is seen to undergo restitution (Lewis 1961). When this
cell ruptures, almost the whole surface is destroyed and the
edges of the membrane are left projecting into the lumen. Such
loose membranes were not seen in the lumen of T. californicus
where secretion is thought to be occurring. Instead, the cell
seems to have extruded into the lumen and then undergoes
decomposition until the plasma membrane is disintegrated and the
cell remnants and products are free in the lumen. Schultz
(1976) believes that extrusion by the blister-like cell (B-cell)
of the amphipod hepatopancreatic caeca, as in the crayfish hepatopancreas (Loizzi 1971), is an active process of apocrine pinching off of the apical complex.

Another controversy, other than whether merocrine or holocrine secretion is occurring, concerns whether the extrusions into the lumen are a result of degeneration of the cells or are a true form of secretion. In insects it is suggested that this is a degenerative process that occurs during starvation (Smith 1968). Alikhan (1969) found the number of extrusions did not increase with increasing maltase activity and so Alikhan did not feel the extrusions were a form of secretion. It was suggested that during starvation the decrease in gut size squeezes the cells out. De Priester (1971) follows this line of reasoning, suggesting that the extrusions are a means of eliminating degenerated cell components that have undergone autophagy. Bunt (1968) found that the degeneration and rupturing of mature type R-cells (storage cells) of the crayfish hepatopancreas has been misinterpreted as secretion, and that extrusion is actually carried out by F-cells (fibrillar cells).

The merocrine and/or holocrine secretion seen in the midgut of T. californicus may be a degenerative process as discussed above. Possible evidence for this is the absorption of lipid by the cells that are later secreted. Studies on animals (Takahashi, Philpott and Miguel 1970; Howse and Welford 1972) and plants (McLean 1968; Schuster, Hershenov and Aaronson 1968) have indicated that one of the characteristics of senescence is storage of lipids. In fact, such a degenerative process may be the means by which the final function of the cell types involved
is enacted. The lipids absorbed previous to this stage are possibly metabolized and passed on to the haemocoel just as in any other absorbing cell.

**Hindgut**

The cells of the anterior hindgut of *Tigriopus californicus*, as in the terrestrial isopod (Vernon, Herold and Witkus 1974), suggest little synthesis is occurring yet the well developed mitochondria suggest an energy requiring function for these cells. The deep indentations of the lumen into the cells and the thinness of the cuticle lining this region of the hindgut also suggest absorption and transport of materials from the gut lumen into the epithelial cells. Faecal pellets are frequently seen in this region of the gut. They may be stored here to take advantage of the last chance to absorb nutrients from the digested remains. Many nutrients are absorbed through the anal vesicle of the wasp (Edson and Vinson 1977). In an ant the faecal fluid contains high levels of enzymes active in the degradation of protein, chitin and starch (Martin 1975).

The occurrence of antiperistaltic activities of the arthropod hindgut, to draw water into the hindgut (Marshall and Orr 1972; Schultz and Kennedy 1976), suggests a function of osmoregulation for this region. In smaller crustacea anal drinking is continuous while in the adults of larger species it occurs in intermittent bursts (Fox 1952). In the brine shrimp osmoregulatory ability of the gut epithelium is implied by the fact that it is continuously taking up hyper-, isohypotonic medium (relative to the haemolymph) making it hyposmotic and taking up water (Croghan 1958). Water uptake and
salt excretion were also found to occur in the Metapenaeus shrimp gut (Dall 1967).

Insect osmoregulation by the hindgut has been studied to a great extent (Berridge and Oschman 1972). The blowfly has cortical cells arranged in the form of four cones projecting into the rectal lumen. These cells function for ion and water transport (Gupta and Berridge 1966). This system has extensive intercellular spaces surrounded by epithelial cells but isolated from the haemocoel. Material in these intercellular sinuses must come either from, or through, the hindgut epithelial cells. The same sort of arrangement of cells, sinuses and junctions occurs in the much studied rectal papillae of the cockroach.

Water regulation of the hindgut involves regulation of one or more ions (possibly potassium). With the aid of energy producing mitochondria which are abundant and an ATPase (Tolman and Steele 1976), the ion is actively pumped into cavities in the cells. In the locust high levels of a sodium-potassium-activated ATPase were present in microsomal fractions of the rectum only (Peacock 1976). Water then enters these cavities from the cytoplasm of the cells thus creating a chain of events with the ultimate source of water being the gut lumen and the ultimate destination of the water being the haemocoel (Smith 1968). The cuticle of the hindgut plays a part in this process by not allowing the passage of large molecules from the lumen into the cell cavities, such molecules are excreted (Oschman and Wall 1969).

In Tigriopus californicus the thin cuticle with its many invaginations penetrating into the epithelial cells and the
presence of mitochondria suggest the possibility of an osmoregulatory process similar, though simpler, to that described above.

The relatively flat basal lamina of the T. californicus anterior hindgut, as in the cockroach, allows ions that are resorbed by the epithelial cells to diffuse away before equilibration is complete (Oschman and Wall 1969). This means that there would not be a proportional flow of water into the rectal pads. The fact that no muscular system was noted in the Tigriopus californicus hindgut adds credence to the theory that osmoregulation through ion transport may occur here. This lack of musculature may allow the necessary recycling of the ions lost from the hindgut epithelia in the osmoregulation process (Oschman and Wall 1969; Noirot and Noirot-Timothee 1976). Studies have shown that a muscle layer between the rectal pads and the haemolymph can act, to some extent, as a physiological barrier to the movement of water and ions (Oschman and Wall 1969).

In a terrestrial isopod the hindgut is also suggested to be involved in water and ion movement and microtubule bundles are believed to function for support of the large cells, movement of water and ions through the cells and aligning the mitochondria (Witkus, Grillo and Smith 1969). In the sugar cane beetle, ion secretion occurs in the posterior midgut (Cheung and Low 1975).

In summary, the T. californicus digestive tract is composed of a cuticularized esophagus, a midgut caecum, an anterior and posterior midgut, and an anterior and posterior hindgut. Table one provides a summary of the cell types, their location,
structure and function. The esophagus has a well developed muscle system which may function to draw food into the gut. The musculature and shape of the esophagus allows for dilation of the esophagus lumen to accommodate large diatoms and other such food. The cells of the esophagus do not possess the organization normally associated with enzyme synthesis and the cuticle would probably inhibit secretion into the lumen of such digestive enzymes. However, some digestion does occur here, probably as a result of enzymes secreted by the midgut and moved forward by antiperistaltic contractions. The ventral esophagus appears to function in the trituration of food. The dorsal esophagus, with its thinner cuticle, appears to be involved in accumulation of electron dense material, possibly storage material.

In the midgut caecum the abundance of cell type three, presence of abundant lipid droplets, great number of invaginations of the epithelial apices, the length of the microvilli and development of the basal lamina suggest this region of the gut functions mainly in absorption of digested nutrients.

The anterior midgut also functions for nutrient absorption but another major role appears to be merocrine secretion. This region of the midgut is unique in having electron dense concretions found in the cell types two and three. It is also unique in having a fourth cell type. This cell type appears to function in lipid absorption.

The posterior midgut functions mainly in secretion, though some absorption is evident. The anterior hindgut probably
functions mainly for osmoregulation and as a last chance at absorbing nutrients from the now forming faecal pellets. Just before excretion, faecal pellets are kept in the heavily cuticulized posterior hindgut. This region of the hindgut may function in compacting the faecal pellets.

It can be seen, therefore, that the overall shape of the digestive tract of *T. californicus* is similar to other copepods. Other arthropods, typically malacostracans, have a hepatopancreas branching off of the midgut. *T. californicus* does not have a hepatopancreas but the functions of this organ are fulfilled by the midgut. The cell types described for a variety of other arthropods from crustaceans to insects occur in *T. californicus*.

Food material, in the form of bacterial cells and diatom frustules, when present in the *T. californicus* gut, occurred in aggregates. One aggregate is found in the lumen of the anterior midgut, another in the lumen of the posterior midgut and a faecal pellet aggregate in the posterior hindgut. Each aggregation of food took the form of an oblong bolus. No matter where in the midgut food material was seen, it was equally digested. It was not until the hindgut that maximum digestion appears to have been completed. These observations along with studies of the gut musculature suggest peristaltic and antiperistaltic contractions have moved food material back and forth in the gut lumen from regions where absorption occurs (midgut caecum and anterior midgut) to regions where digestion occurs (anterior midgut and posterior midgut).

In most cases the gut lumen was void of food material of
any form. It seems probable that the shock of fixation or handling causes the copepod to eject the contents of its gut.

Continuous uniculture feeding studies would give an indication of the food preferences and growth patterns of this copepod. Radioactively labelling the lipids and/or proteins of food sources of the copepod would be an ideal way to find, fairly conclusively, the assimilation ability and rates. Pulse labelling with such radioactive food would allow one to follow the passage of food through the gut. Such a pulse labelling method would also give an indication of absorption rates and where in the gut each of these nutrients was being absorbed.
KEY FOR FIGURES

A-anus
A1-first antenna
A2-second antenna
Ah-anterior hindgut epithelial cells
AM-anterior midgut epithelial cells
Ap-appendage
B-basal lamina
c-cuticle
Ct-cephalothorax
D-diatom frustule
E-esophagus epithelial tissue
EC-excreting (ed) cell
ER-endoplasmic reticulum
ES-egg sac
Ex-external environment
f-faecal material
g-golgi
H-haemocoel
L-lumen
l-lipid
M-muscle
m-mitochondria
MC-midgut caecum epithelial cells
Mc-circular muscle
ML-longitudinal muscle
Mt-microtubules
Mv-microvilli
MV-multivesicular bodies
N-nucleus
No-nucleolus
NT-nervous tissue
O-ovary
Od-oviduct
P-peritrophic membrane
P1, 2, 3, 4-pereiopods 1, 2, 3, and 4
Ph-posterior hindgut epithelia
PM-posterior midgut epithelia
R-rostrum
r-ribosomes
RER-rough endoplasmic reticulum
S-secreted material
SER-smooth endoplasmic reticulum
Tj-tight junction
V-vesicle
Z-Z band of muscle sarcomeres

1-cell type one
2-cell type two
3-cell type three
4-cell type four

*-region where holocrine secretion may have occurred.
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Figure 1a. Scanning electron micrograph of an adult male *T. californicus*. Note the enlarged tip of the first antennae.
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Figure 1b. Scanning electron micrograph of an adult female T. californicus. Note the egg sac cradled by the posterior pereiopods.
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Figure 1c. Low magnification light micrograph of a whole mount of a mating pair of adult *Tigriopus californicus*.

Figure 2. Sagittal section of *T. californicus*. Arrows indicate the position of transverse sections in the plates indicated. The overall shape of the digestive tract is evident as is the cuticle lining the esophagus and hindgut.
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Figure 3. The esophagus surrounded by muscle proximally and haemocoel distally.

Figure 4. Midgut caecum regions showing abundant vesicles undergoing possible secretion.

Figure 5. This micrograph shows the esophagus entering the anterior midgut in the region where the midgut caecum opens into the anterior midgut.

Figure 6. The midgut caecum and esophagus is seen in this micrograph to have fused with the anterior midgut. Note the cuticle of the esophagus ends abruptly to give way to microvilli of the midgut cells.
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Figure 7. The posterior midgut, cells in this region have fewer vesicles that are distinctly smaller than those of the anterior midgut. Note the different cell sizes, the larger cells line the ventral surface of the gut.

Figure 8. The anterior hindgut epithelia, note the rather thick and dense cells lacking cuticle. This region of the hindgut has little muscle tissue around the digestive tract but it is surrounded by the basal lamina and the haemocoel.

Figure 9. A portion of a sagittal section of the transition area between the anterior and the posterior hindgut. Note the thickening of the cuticle and absence of epithelial tissue.

Figure 10. The posterior hindgut as it appeared when dilated. The region appears to lack an epithelial lining but is lined by relatively thick cuticle.
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Figure 11. The entrance of the esophagus into the anterior midgut of the digestive tract is shown in this sagittal section. Note cuticle of esophagus ends abruptly where microvilli of midgut begins.

Figure 12. A cluster of cell type one not extending to the lumen are seen in this micrograph. Note the large nuclei in these cells.

Figure 13. This light micrograph shows cell types one, two and three. Cell type two and three are seen to extend from the basal lamina to the lumen.

Figure 14. Cell type two, being extruded into the anterior midgut lumen. Cell types one and three can also be seen in this figure. Figure 13 and 14 may be taken as evidence for a process of secretion of cell type two.

Figure 15. The epithelial cells of the posterior midgut and the haemocoel directly below the epithelial basal lamina are seen in this micrograph. The cells are smaller and appear pseudo-stratified. Cell types one, two and three are still present.
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Figure 16. Esophagus lumen and epithelial tissue surrounded by circular muscle. Esophagus lumen takes on an 'H' appearance.

Figure 17. Esophagus as it enters anterior midgut. Note lack of circular muscle and unidentified multivesiculate bodies in epithelial cells.

Figure 18. Higher magnification of region next to esophagus cuticle showing longitudinal and cross sections of associated microtubules.
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Figure 19. Midgut caecum epithelial cells. The abundant vesicles of cell type three are evident as are the well developed golgi bodies of cell type two.

Figure 20. Exocytosis of cell type three in midgut caecum. Microvilli in both longitudinal and cross section are shown.

Figure 21. Higher magnification showing interdigitated basal lamina of midgut caecum.

Figure 22. Microvilli of the midgut caecum and evidence for merocrine or holocrine secretion are shown in this micrograph.

Figure 23. Three type one cells are shown lying at the basal lamina. Note that they do not reach the lumen.

Figure 24. Cell type two (on right) and cell type three (on left) are shown. Note large vesicles of cell type three and smaller ones of cell type two.
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Figure 25. In the anterior portion of the anterior midgut, deep furrows extend from the lumen and almost reach the basal lamina of the cells. Note secretion in the upper right corner of the micrograph. Also note the cell type four in the lower mid portion of the micrograph.
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Figure 26. Although cell types two and three can be distinguished in this region of the gut, their differences in overall electron density are less obvious. The fourth cell type of the anterior midgut is enclosed in the circle outlined by a dark line. Longitudinal muscle is seen in association with the invaginations of the basal lamina. In this micrograph multivesicular bodies can be seen
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Figure 27. This higher magnification micrograph shows the multivesicular bodies of the anterior midgut. Note that many vesicles, often with lamellate structures, make up one body.
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Figure 28. The anterior midgut is seen, in this micrograph, to possess evaginations associated with cells of type one. Cell types two and three are less differentiated from each other. Cell type two is less electron dense and cell type three is more electron dense than in the midgut caecum. Note the longitudinal muscle associated with the invagination of the basal region of the cells.

Figure 29. This is a higher magnification of the cell type four of Figure 25. Note the great number of vesicles containing electron dense secretory products. Vesicles at all stages of maturation are evident. The vesicles may be: 1. absorbed lipid, 2. zymogenic, or 3. endocrinal. These vesicles are very similar, in size (300-400μm) and appearance, to the gastrin-producing cells of the stomach in higher organisms.

Figure 30. In the posterior midgut the basal lamina shows fewer invaginations and evaginations and the microvilli, often only associated with cell type two, are shorter and less regularly arranged. Note the circular muscle at the base of the cells.

Figure 31. In this micrograph cell type two, in the posterior midgut, are seen to send 'arms' to each other. Vesicles containing electron dense materials are common in these cells.
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Figure 32 and 33. These two micrographs are evidence of holocrine secretion in the posterior midgut. The increased number of cell type one also adds credence to the theory that holocrine secretion is occurring. Note the disruption of the microvilli where this form of secretion is occurring and the close position of cell type one in both micrographs.
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Figure 34. In the anterior hindgut the microvilli become shorter and more scarce. Rough E. R. is abundant as are some vesicles. The basal lamina is less invaginated and the cells are significantly smaller.

Figure 35. In the posterior hindgut the microvilli have been totally replaced by a thin cuticle and faecal material has replaced the more recognizable food material found more anteriorly in the digestive tract lumen.

Figure 36. This electron micrograph shows the transition from the finer cuticle to the denser cuticle in the most posterior regions of the posterior hindgut. The epithelial tissue in these regions is minimal.

Figure 37. In the region of the anus the cuticle is apparently identical in structure to the cuticle surrounding the outer regions of the body.

Figure 38. This is a higher magnification of the region of the cuticle indicated by the dashed box. Note the electron transparant regions lying next to the epicuticle.
| ONE | PMDG | -does not reach lumen |
| AMDG | -found in clusters of 2-4 cells |
| MDGC | -basal lamina infoldings isolate this cell type but do not penetrate cytoplasm |
| -some mitochondria |
| -minimal ER or golgi |
| -varying abundance of ribosomes |
| Function | -replace cells worn away or lost during secretion |
| References to Similar Cells in Other Arthropods | -van Weel 1955 |
| | -Pillai 1964 |
| | -Martenstein 1968 |
| | -Sud 1968 |
| | -Clifford and Witkus 1971 |
| | -de Priester 1976 |
| | -Loizzi 1971 |
| | -Schultz 1976 |

| TWO | PMDG | -reaches lumen and has microvilli |
| AMDG | -has regular invaginations of basal lamina |
| MDGC | -randomly dispersed mitochondria |
| -rough ER and loose ribosomes produce a very dense cytoplasm |
| -dilated golgi bodies |
| -exocytotic vesicles with electron opaque centers |
| -some lipid absorption |
| Function | -synthesis of enzymes |
| References to Similar Cells in Other Arthropods | -Dakin 1988 |
| | -Lowe 1935 |
| | -Reddy 1938 |
| | -van Weel 1955 |
| | -Pillai 1960 |
| | -Fahrenbach 1961 |
| | -Lewis 1961 |
| | -Clifford and Witkus 1971 |
| | -Loizzi 1971 |
| | -Marshall and Orr 1972 |
| | -Schultz 1976 |

| THREE | MDGC | -reaches lumen and has microvilli |
| AMDG | -invaginated basal lamina |
| PMDG | -randomly distributed mito. |
| -basal lamellate rough ER. |
| -numeros golgi bodies |
| -small and large vesicles seen throughout the cell |
| -lipid vesicles apically in anterior midgut contain electron dense multivesiculate bodies |
| Function | -mainly absorption |
| References to Similar Cells in Other Arthropods | -Dakin 1988 |
| | -Lowe 1935 |
| | -van Weel 1955 |
| | -Pillai 1960 |
| | -Fahrenbach 1961 |
| | -Lewis 1961 |
| | -Smith 1968 |
| | -Sud 1968 |
| | -Clifford and Witkus 1971 |
| | -Loizzi 1971 |
| | -Marshall and Orr 1972 |
| | -Schultz 1976 |

| FOUR | AMDG | -reaches lumen and has microvilli |
| -minor invaginations of basal lamina |
| -mitochondria slightly more abundant basally |
| -minimal rough ER. |
| -small and undilated golgi bodies |
| -abundant supply of small electron dense vesicles apically |
| Function | -appears to be lipid absorption |
| References to Similar Cells in Other Arthropods | -Dakin 1988 |
| | -Lowe 1935 |
| | -van Weel 1955 |
| | -Pillai 1960 |
| | -Fahrenbach 1961 |
| | -Lewis 1961 |
| | -Smith 1968 |
| | -Sud 1968 |
| | -Clifford and Witkus 1971 |
| | -Loizzi 1971 |
| | -Marshall and Orr 1972 |
| | -Schultz 1976 |

*in order of decreasing frequency

-AMDG=anterior midgut
-MDGC=midgut cecum
-PMDG=posterior midgut
LITERATURE CITED


Beams, H. W. and E. Anderson. 1957. Light and electron microscope studies on the striated border of the intestinal


Gupta, B. L. and M. J. Berridge. 1966. Fine structural organization of the rectum in the blowfly, Calliphora erythrocephala (Meig.) with special reference to connective tissue, tracheae and neurosecretory innervation in the rectal papillae. J. Morph. 120: 23-82.


Rigdon, R. H. and D. J. Mensik. 1976. Gastrointestinal tract


Van Weel, P. B. 1955. Processes of secretion, restitution, and
resorption in gland of mid-gut (glandula media intestini) of *Atya spinipes* Newport (Decapoda-Brachyra). Physiological Zool. 28: 40-54.


