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STUDIES ON THE SHRIMP *CARIDINA LAEVIS* (HELLER)

1. The Digestive System

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INTRODUCTION

EXCEPT for a few papers on the structure of the foregut and the physiology of digestion in a few forms, no comprehensive account exists on the detailed anatomy of the alimentary canal and associated glands of the Crustacea of India. Patwardhan (1935) has made a comparative study of the foreguts of quite a number of Decapods including eighteen species of Caridea. Later (Patwardhan 1937) the digestive system of *Palaemon malcolmsonii* was described by him. The physiology and cytology of digestion in *Paratelphusa hydrodromus* was studied by Reddi (1937, 1938). A detailed account of the anatomy of the digestive system and the physiology of digestion and assimilation in *Nephrops norvegicus* was given by Yonge (1924). The nature and permeability of the chitinous layer of the foregut in Decapoda have been elucidated by him in 1932. The anatomy and histology of the digestive system of *Galathea* was worked out by Pike (1947). Jacobs (1928) and Weel (1955) have made important contributions to the cytology and physiology of the digestive diverticula in *Astacus leptodactylus* and *Atya spinipes* respectively. In the present paper an attempt is made to describe the detailed anatomy and histology of the digestive system of a Caridean shrimp. *Caridina laevis* was chosen in view of its easy availability and extreme hardiness.

MATERIAL AND METHODS

Caridina laevis is abundant in ponds and lakes with plenty of vegetation and clean water. Specimens were collected with the help of a common ring net made out of mosquito netting. The animals were reared in a 20″ × 10″ × 10″ glass aquarium containing pond water with vegetation (*Hydrilla* and *Elodea*) and sand. Tank water was found most suitable though tap water had no visible detrimental effects. The aquarium was installed in such a way that direct sunlight was available for about a couple of hours every day and the water was changed once in four days.

The gross anatomy of the digestive system and the structure of the stomach were studied by micro-dissection under a stereoscopic binocular microscope in artificial illumination. Histology was studied by sectioning the different regions of the animal. The exoskeleton being soft enough, animals were easily cut by the methyl-benzoate-celloidin technique (Pantin 1948). Different stains and techniques were employed which are dealt with in the appropriate places.

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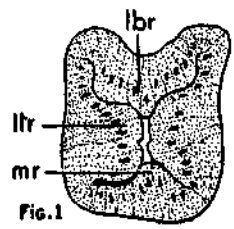
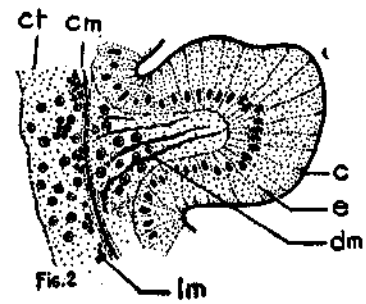
1. The Foregut

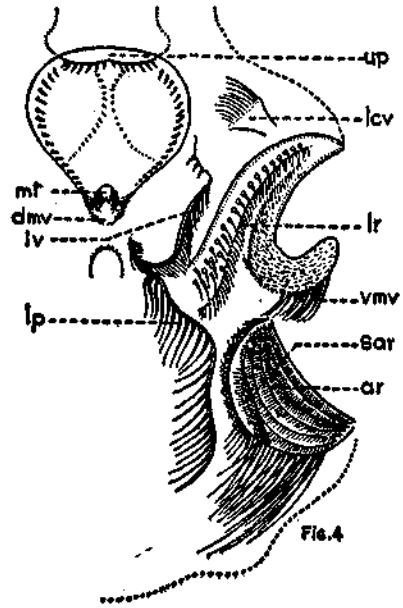
The mouth is a longitudinal narrow slit opening into the food basin (the space enclosed by the maxillipeds, Manton 1928). The oesophagus leading from it is a short vertical tube 260μ long and 120μ wide maximally.

Structure of the oesophagus (Fig. 1)

The oesophagus is squarish in section and presents three ridges inside. The labral ridge (lbr) is continuous with the labrum. The lateral ridges (ltr) are broader.







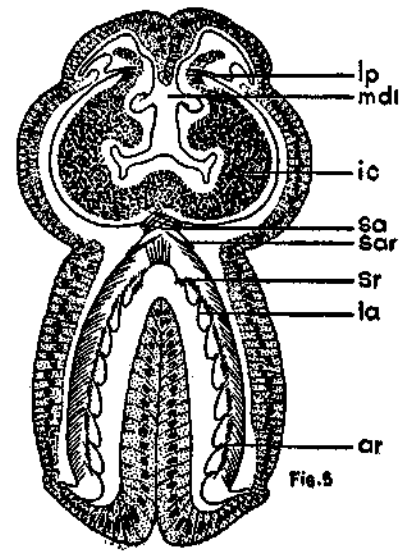


Fig. 1. C.S. of oesophagus through its distal part.
Fig. 2. A portion of the oesophageal wall.
Fig. 4. Inner view of cuticular wall of the stomach after cutting through the median ventral line of the pyloric stomach.
Fig. 5. A vertical section through the anterior part of the pyloric stomach.

The hind wall of the oesophagus is devoid of a ridge proximally, although, distally a small ridge, the metastomal ridge (mr) makes its appearance. The lumen of the oesophagus is X-shaped because of these ridges which fade out imperceptibly where the oesophagus opens into the stomach.

Histology (Fig. 2)

The wall of the oesophagus is lined internally by a thin layer of cuticle (c). Outer to this is the epithelial layer (e) made up of columnar cells. The cells are about 55μ long with clear cytoplasm and small oval, basal nuclei. Surrounding the epithelium is a connective tissue envelope (ct) which extends into the core of the oesophageal ridges. Circular (cm), longitudinal (lm) and dilator muscles (dm) of the oesophagus are found in close association with the connective tissue layer.

A few glands have been observed in the wall of the oesophagus. Their structure is quite similar to that of the tegumental glands and will be described in a subsequent communication on the integument.

Structure of the Stomach (Figs. 3, 4, 5 & 6)

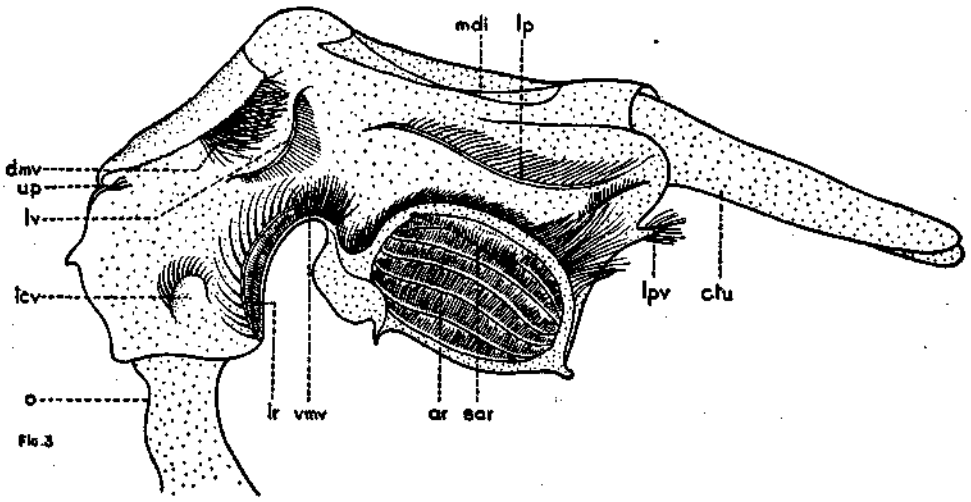


Fig. 3. Lateral view of stomach after removal of muscles and other soft tissues.

METHODS

Stomachs from starved animals were treated with a weak solution of KOH and the soft tissues teased off under water. The structure of the cuticular lining was studied in reflected light under the low power of the microscope. These observations were confirmed on sectioned material and exuviae.

Out of the two divisions of the stomach *viz.* cardiac and pyloric, the latter is larger being 0.7 mm. long and 0.5 mm. high in an adult female specimen, while the cardiac stomach is 0.5 mm. long and 0.3 mm. high. The median dorsal wall of the cardiac and pyloric divisions is depressed into their lumen except at the region of the cardio-pyloric junction.

The proximal part of the cardiac stomach is rectangular clearly demarcating the oesophageal portion(o) from it. Beyond and on either side of the opening of the oesophagus, the cardiac walls bear two roughly triangular valves (lcv). The fore end of the cardiac roof bears a faintly notched median transverse plate (up) abutting into the lumen and bearing a few short bristles at its free margin. Between this and the cardio-pyloric opening, the cardiac roof is heart-shaped and depressed. Its tapering hind end is infolded backwards below the roof of the cardio-pyloric opening. This bears a tubercle with two small teeth at its tip (mt) which might correspond to the median tooth of other decapods. On either side of this median tooth are two clusters of long bristles, extending into the cardio-pyloric opening. This is the dorsal median valve (dmv). The floor of the cardiac stomach is convex and consists of a median plate bearing two almost parallel ridges, the longitudinal ridges (lc) bearing two or three rows of bristles directed towards the cardio-pyloric opening. The plate has a coat of velvetty bristles. The hind end of the median plate bears a cluster of short bristles which constitutes the median ventral valve (vmv). The lateral wall of the cardio-pyloric opening has a crescentic thickening carrying bristles and constituting the lateral valve (lv).

The cardiopyloric opening leads into the pyloric stomach whose roof is also depressed into its lumen. The lateral wall of the pyloric stomach has a longitudinal thickening, the lateral pyloric thickening (lp), which extends to its hind end bearing long backwardly directed bristles. Below this it is folded inwards forming the supra-ampullary fold (sa) which divides the pyloric stomach into a dorsal and a ventral chamber. The median part of the floor of the lower chamber of the pyloric stomach is raised into the shape of a median biconvex fold, the interampullary fold (ia) which divides it into two chambers, the ampullae, appearing in cross section like an inverted V. The summit of the interampullary fold and the supraampullary folds bear inumerable long bristles in a criss-cross manner so that the slit-like opening into the lower chamber of the pyloric stomach is converted into a fine sieve thereby preventing the larger particles of food in the dorsal chamber from entering the ampullae and congesting its narrow lumen. The inner wall of each ampulla *viz.* the outer wall of the interampullary fold is raised into eight longitudinal ridges (ar). From each of the lower ridge extends a row of fine silky bristles to the ridge above. The uppermost ridge has a serrated margin (sr) in place of bristles. The outer wall of the ampulla is free from ridges but bears numerous bristles in an orderly fashion. Below the supraampullary ridge, it bears a row of short and stout bristles (sar). The two ampullae diverge anteriorly and lead into a common space, behind the ventral median valve. The opening of the ampullae into the midgut, behind, is guarded by a pair of small lateral valves (lpv).

The roof of the pyloric stomach presents a rhomboid depression (rhd). The fore end of the rhombus involutes below the roof of the cardio-pyloric opening has a pointed double-walled pocket, almost touching the similar semicircular involution from the hind end of the cardiac stomach. On the longitudinal longer diagonal of the rhombus is a narrow deep invagination of the cuticular wall, the median dorsal pyloric infold (mdi). The postero-lateral sides of the rhombus expand on either side into a pair of wings (w). Leading from the upper division of the pyloric stomach are two long half-tubules which together function as a conveyor tube (ctu). The coarser contents of the pyloric stomach are conducted far back into the midgut without their obstructing the openings of the digestive divertcula and the midgut caeca which open immediately behind the pyloric stomach.

Histology

The cuticular lining of the stomach is thicker than that of the oesophagus, but the formation of ossicles does not take place unlike in most other decapods. The epithelium is unilaminar or polylaminar in which case it is thick and lines some of the depressions of the cuticular lining. No muscle fibres incorporated in the stomach wall have been identified.

Gastric Muscles (Fig. 7)

The list of extrinsic and intrinsic muscles, based on the classification of Mocquard (1883) is given below. As far as possible the same terminology has been followed.

A. *The Extrinsic muscles*

1. Anterior gastric muscle.....(ag)
2. Posterior gastric muscle.....(pg)
3. Anterior superior dilator muscle.....(od)
4. Anterior cardiac dilator muscle.....(ac)
5. Anterolateral cardiac dilator muscle.....(cad)
6. Anterior oesophageal dilator muscle.....(aod)
7. Lateral oesophageal dilator muscle.....(lod)
8. Posterior oesophageal dilator muscle.....(pod)
9. Ventral pyloric dilator muscle.....(pd)

B. *The Intrinsic Muscle*

1. Lateral pyloric muscle.....(lp)
2. Longitudinal muscles of the ampulla.....(la)
3. Transverse pyloric muscle.....(tp)
4. Lateral cardiac muscle.....(lc)
5. Tranverse cardiac muscle.....(tc)
6. Posterior inferior cardiac muscle.....(pc)
7. Anterior cardiac muscle.....(ac)

General considerations on the stomach and gastric muscles

Patwardhan (1935) has worked out the structure of the stomach of numerous decapods. The account includes a cursory reference to that of *Caridina brachydactyla* also. It is remarkable that the stomach figured and described by him differs obviously from that of *C. laevis*.

In spite of the fact that some members of the group Penaeidea exhibit complex types of gastric armatures, the Penaeidea is supposed to possess the simplest gastric structure. Bonnier (quoted by Calman 1909) has studied the simple gastric structure of *Cerataspis*, a penaeid. *Caridina* and other members of the tribe Caridea, characterised by the absence of a gastric mill, fall into a group by themselves under Decapoda. The cardiac stomach and the dorsal section of the pyloric stomach of these bear a few important resemblances with those of *Cerataspis*. These are briefly, the median dorsal depression of the cardiac stomach bearing the median tooth, the median ventral plate of the cardiac stomach bearing two parallel ridges, the lateral wall of the pyloric chamber bearing bristles, which in *Caridina* are restricted to

form the lateral pyloric bristles, the posterior part of the pyloric stomach bearing long extensions (the lappets in *Cerataspis* and the conveyor tubes in *Caridina*) and the two smaller valves below these. It is obvious that the stomach of *Caridina* shows greater complexity of organisation than that of *Cerataspis* mainly in the presence of the anterior cardiac valves and the lateral valves (which in *C. brachydactyla* almost assume the proportions of the lateral teeth), a median tooth and the single row of lateral pyloric teeth. Patwardhan (1935) has concluded that the absence of a gastric mill in the members of Caridea is due to its secondary suppression represented by degrees in the series *Caridina, Hippolyte* and *Virbius*. This is based on Borradaile's (1907) classification attributing the lowest phylogenetic position to the Penaeidea under Decapoda and the fact that *Cerataspis*, a penaeid, has the simplest gastric armature. But many penaeids have a complex type of gastric structure and hence, until a very thorough comparative account of the stomach of the various decapods is undertaken with a view to elucidating their phylogenetic relationships, the above conclusion of Patwardhan could only be treated as tentative.

There is a paucity of literature on the musculature of the stomach of decapod Crustacea. On comparing the musculature of stomachs with gastric mill with that of *Caridina*, the following conclusions may be drawn. In being devoid of a gastric mill, the gastric musculature of *Caridina* is prone to much variation if not retrogression. The extrinsic muscles have suffered more in this process. The proportions of the more important muscles are reduced and the less important ones even fail to be represented. If the view of Patwardhan is accepted, this must be due to their secondary suppression consequent on the loss of the gastric mill. It is noteworthy that the intrinsic muscles have not changed much ; in fact new ones are represented like the longitudinal intrinsic muscles of the ampulla and the transverse pyloric muscle. What corresponds to the posterior inferior cardiac muscle of decapods extends still further posteriorly and connects the front extremities of the pyloric ampullae. All these would imply the development of new muscles to the pyloric part of the stomach which is outstanding in *Caridina* by its size and organisation.

The working of the stomach and the course of food

During feeding the movements of the stomach may be observed through the transparent cuticle, slightly obscured by the brownish digestive diverticula. The muscles being quite transparent, their individual role in bringing about the desired movements cannot be clearly analysed. The movements of the stomach are of a particular type which are repeated at an average frequency of 32 times per minute. The movements may be analysed in the following order: (1) Lateral walls of the cardio-pyloric junction are apposed. (2) Roof of the cardiac stomach is pulled forwards, slightly narrowing the chamber. (3) Floor of the cardiac stomach exhibits jerky movements backwards and upwards. (4) Two ampullae also pulsate synchronously. The muscles which effect these four types of movements are respectively (1) the transverse pyloric muscles (2) the anterior gastric muscles together with the anterosuperior dilator muscles (3) lateral cardiac muscles and (4) the lateral pyloric muscles together with the longitudinal muscles of the ampulla. The active rôle of the anterior gastric muscles in the contraction of the stomach is disputed. It has been shown to take an active part in forms with a well developed gastric mill (Mocquard 1883 ; Pearson 1908 ; Patwardhan 1935). Huxley (1880) holds that a coordinated working of the anterior and posterior gastric muscles brings about the movements. The rôle of anterior gastric muscle has been questioned by Reddi (1935, 1937) according to whom the posterior gastric plays the important rôle. However this view could not be corroborated in *Caridina laevis*.

The cardiac stomach is seldom found to be completely filled with food particles. The jerky movements of the cardiac floor with its armature resemble the movements of the molluscan radula and transfer the food backwards to the cardio-pyloric opening. When the lateral walls of the cardio-pyloric opening are apposed there is a slight backward pull due to the contraction of the anterior bundle of the lateral cardiac intrinsic muscle. As a result of this the lateral valves of the cardio-pyloric opening play back and forth, backwards when the walls are apposed and forwards when they recede. These movements alternate with those of the median dorsal valve. In other words, when the lateral valves appose the median valve moves forwards and *vice versa*. The food is seen to fill the pyloric stomach completely. It passes on either side of the dorsal median pyloric infold as two streams. Fine particles alone enter the ampullae, which in sections appear as a fine suspension. The two streams of coarser materials in the upper chamber join together behind and are conducted through the conveyor tube far beyond, into the midgut.

The Digestive Diverticula (*Hepatopancreas*)

Methods

Because of heavy secretory activity of the digestive diverticula, the more general types of fixatives do not give a vivid picture of their detailed structure without creating artefacts. Champy's fixative as modified by Weel (1955), Carnoy's and Regaud's fluids were used. Glands fixed in corrosive sublimate-alcohol were also made use of in this study though the results were not good enough. Staining was done in Heidenhain's Azan, Delafield's haematoxylin and Heidenhain's haematoxylin.

The digestive gland consists of numerous blindly ending tubules about 60 μ across, filling up most of the available space inside the cephalothoracic region. There are two main lobes of the gland on either side of the cardiac stomach filling up the thoracic blood sinus. Each lobe consists of a cluster of diverticula which unite to form a number of secondary ducts. The secondary ducts collect together to form the main duct. The two main ducts join together and open into the gut immediately behind the pyloric stomach on its ventral aspect.

Histology

Each tubule of the gland is enclosed in a thin envelope of connective tissue (Fig. 8, ct.) which has numerous extensions which fasten the tubules together and attach them to the surrounding structures. The connective tissue layer is traversed by two types of smooth muscle fibres, the longitudinal and circular, both taking a sky-blue tint in Heidenhain's Azan. The circular muscle fibres are arranged regularly at 10μ intervals and seen to be connected together by a faint network of smaller fibres. The longitudinal fibres are less pronounced and fewer in number which is at variance with the findings of Pump (1914) and Yonge (1924). Beneath the layer of connective tissue is a thin structureless membrane, and then the glandular epithelium of the digestive diverticula. Histologically an apical young region (about 45μ) may be marked off in each tubule representing the embryonic zone (ez). This zone has small, undifferentiated columnar cells measuring about 14μ by 4μ. Nuclei are spherical with more than one nucleolus and a few clumps of chromatin. Most of these cells possess smaller bodies lying distal to the nucleus, *viz.* the parasomes of Jacobs (1928) and minute vacuoles. Mitosis has been observed only very rarely in this zone unlike in *Astacus* (Jacobs 1928). The embryonic zone is followed by the

short transitory zone (tz) where cells are better defined, the cytoplasm slightly spumoid and the nuclei clearly basal. Beyond the transitory zone, the epithelium consists of three types of cells, the Extrusion cells, the Dark cells and the Light cells.

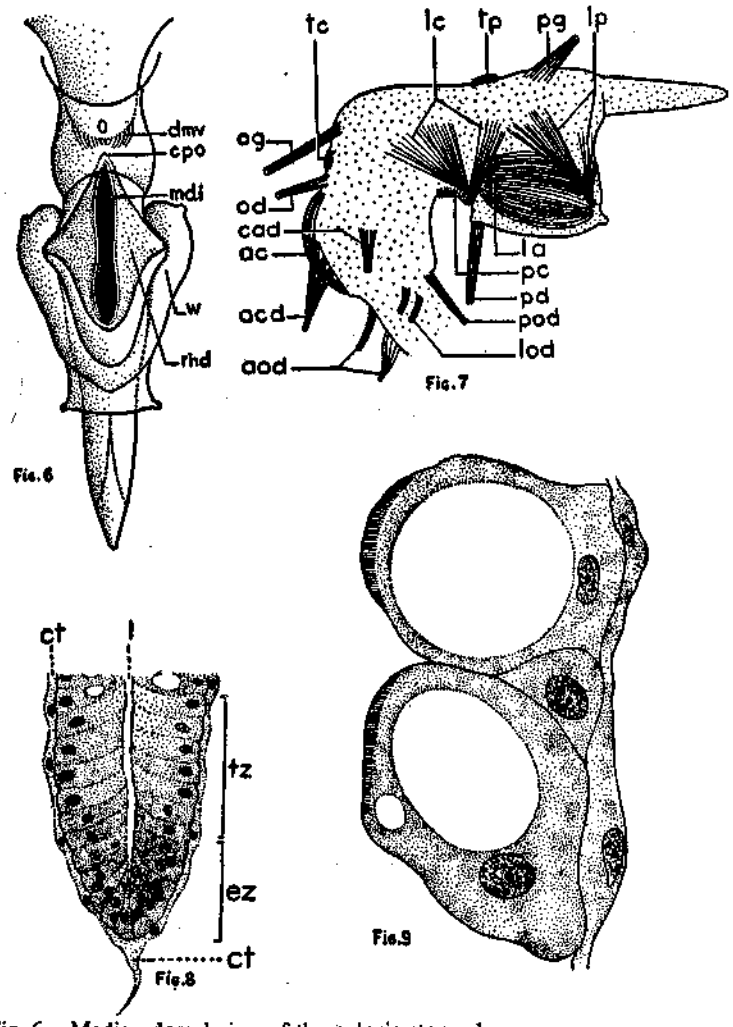


Fig. 6. Median dorsal view of the pyloric stomach.
Fig. 7. Lateral view of the stomach showing the extrinsic and intrinsic muscles.
Fig. 8. L.S. through a tubule of the hepatopancreatic gland.
Fig. 9. Two extrusion cells and a replacement (middle) cell.

Extrusion cells (Fig. 9)

These measure about 35μ by 16μ. A ripe extrusion cell has a large vacuole and a basal nucleus which is crescentic or ellipsoidal with a centrally placed nucleolus. The length of the cell is often determined by the extent of the vacuole and often bulges into the lumen of the tubule. Material fixed in modified Champy's fluid

demonstrates the presence of a faint coagulum and rarely a few crystalloids inside the vacuole. Younger extrusion cells have smaller vacuoles and thicker distal pads of cytoplasm. The apical part of the cytoplasm exhibits faint striations and is better stainable.

Dark Cells

Dark cells have the most life-like appearance in glands fixed in modified Champy's fluid and are rendered markedly more stainable. They (Fig. 10) are as large as the extrusion cells and have larger and more stainable nuclei with excentric nucleoli and numerous clumps of chromatin. The parasomes are often present. Cytoplasm is faintly frothy and presents minute fibrils extending from the base to the apex of the cells. In other fixatives, the dark cells present a different appearance. In Carnoy's fluid the cytoplasm, especially the apical half shows a conglomeration of coarse filament-like structures which are strongly chromophilic. This has been interpreted by Weel (1955) in *Atya spinipes* as artefacts resulting from protein precipitation, representing the typical 'ergastoplasm.' In Heidenhain's Azan, the cytoplasm of the dark cells (Fig. 11) exhibits a pronounced accumulation of dark granules appearing as rings or bodies of other shapes which might indicate a phase of formation of the secretory substance. Thomas (1948) has demonstrated certain azocarminophilic granules inside nerve cells which according to him represent the Golgi spheroids. Though the fixatives employed here are different, the granules and spheroids appear quite alike. Moreover spheroids with an outer more stainable pellicle has been observed in the gland cells vitally stained with neutral red in the present form. This also is in agreement with the findings of Thomas as representing the Golgi complement. In materials stained in Heidenhain's iron haematoxylin, however, this system was not observed.

Light Cells

These (Fig. 12) are strictly columnar cells, narrower than the other cell types described. Nuclei are small, central or partly basal. The cytoplasm is less stainable than the dark cells with strong tendency to vacuolisation and free from any signs of striation.

Smaller cells which measure half the size of the extrusion cells are met within the epithelium which are the replacement cells (middle cell in Fig. 9).

Secretion and Restitution Cycle

METHODS

Freshly collected animals were subjected to complete starvation for 72 hours. In the earlier period of this, water was changed every 15 minutes as the animals are coprophagous. When the gut was rendered empty a few animals were dissected and the digestive diverticula fixed. The other animals were fed on mud and algal filaments. They were killed at regular known intervals and the hepatopancreatic tubules fixed and sectioned. Fixatives employed were Carnoy's fluid and modified Champy's fluid. Both gave tolerably good pictures of the phases of secretory activity of the gland. Stage counting of the various cell types during the different phases of feeding was carried out.

In the hepatopancreas of a normally fed animal, the extrusion cells were more numerous than the dark cells. The lumen of the tubules fixed in Carnoy's fluid

and modified Champy's fluid showed a granular coagulum. The coagulum was absent in animals subjected to 72 hours' starvation. Here the extrusion cells and dark cells showed the same frequency as in a well-fed animal. The extrusion vacuoles were small. In the fed animals after 30 minutes, a faint reduction in the number of extrusion cells has been observed. The vacuoles were much larger and pressed against each other rendering the lumen irregular. In tubules fixed one hour and fifteen minutes after feeding, the frequency of extrusion and dark cells had gone down. However an increase in the number of replacement cells was noticed. Little change had taken place in tubules fixed after one hour and forty-five minutes but for the definite increase in the frequency of dark cells. Their cytoplasm exhibited a uniformly chromophilic granular appearance and the fibrillar nature was represented only sporadically. At 2 hours and 15 minutes after feeding the dark cells demonstrated the fibrillar structure described earlier as the ergastoplasm. A few lingering vacuoles still remained. By about 2 hours and 45 minutes after feeding, there was a marked domination of the extrusion cells over the dark cells. Vacuoles of various sizes, large and small, were seen. The dark cells have either not changed or there was slight reduction. Tubules fixed still later, after 30 minutes, showed an increase in the extrusion cells. Most vacuoles were large, apical and apparently about to be extruded. The lumen of the tubule appeared to be bounded by an array of vacuoles. Dark cells were scarce comparatively. The ripe vacuoles ceased to exist in tubules fixed after four hours and 15 minutes. Smaller vacuoles were abundant and these with the vacuoles that are developed at this stage by the light cells bestowed a 'bubbling' appearance to the epithelium, obscuring the cellular details and the boundary of the lumen. The bubbling appearance was lost and the boundary of the lumen once more established at about five hours after feeding. The secretory vacuoles of the extrusion cells were not represented except for a few lingering stray ones whose extrusion has been delayed probably by a lack of feeding stimulus. The dark cells may be described to be in the ascendant. In another three hours, the extrusion cells were also on the increase.

With the initiation of feeding, the number of extrusion cells is on the decline indicating a phase of extrusion. This phase lasts for two hours and a half after which the restitution phase commences. This reaches its mode between two hours 40 minutes and four hours 15 minutes. Following this, at about five hours after feeding the second extrusion phase sets in and the next phase of restitution appears to be attained in another three hours. The chronological disposition of these phases is at variance with the findings of Weel (1955) in *Atya spinipes* where the number of extrusion cells reaches its peak during the first 30 minutes after feeding has started, followed by a minimum at four hours. A second maximum is attained at 12 hours. Synchronous with the initiation of feeding there is a sharp rise in the number of extrusion cells in *Atya* while in *Caridina* they are on the decline. The secretory cells of the digestive diverticula are polyphasic, to use the terminology of Hirsch (1931). The secretion is merocrine unlike that of *Astacus* (Hirsch and Jacobs 1930).

The phenomenon of secretion and restitution of the gland cells of the hepatopancreas has been elucidated by Hirsch and Jacobs (1929, 1930), Jacobs (1928) in *Potamobius leptodactylus* and Weel (1955) in *Atya spinipes*. In *Potamobius*, Jacobs has observed a direct transformation of the light cells and dark cells into extrusion cells. Such a transformation of the dark cells into extrusion cells is not apparently

found in *Atya*. In *Caridina*, the stage counting shows that the dark cells and extrusion cells at any given time show an inverse relation, i.e., when one has a high frequency the other has a low one. The dark cells get directly transformed into the extrusion cells unlike in *Atya*. The smaller cells met within the epithelium are the replacement cells described in various other Crustacea (Yonge 1924, Pike 1947, etc.). The replacement cells in *Nephrops* are thought to take the place of old discarded cells. In tubules fixed one hour after the initiation of feeding, the count of the extrusion cells has gone down with the dark cells in *Caridina*. The increase in the replacement cells at this stage may be correlated with the decrease in the number of extrusion cells. The replacement cells may thus be derived from the extrusion cells after their phase of extrusion. Mitotic figures were not observed either. The transformation of the light cells into the extrusion cells cannot be ascertained as they exhibit little change in count during the phases of secretion and restitution.

Mechanism of extrusion

Stained sections of the digestive diverticula suggest clearly a discharge of vacuolar contents of the extrusion cells by rupture. The lumen of the hepatopancreatic tubule contains a lightly staining coagulum possibly formed by the secretory contents and fine food materials. Reddi (1938) has shown in *Paratelphusa* that the zymogen region of the secretory cells either ruptures or dome-shaped structures are detached *en masse* into the lumen. Such cytoplasmic extrusions are met with in *Caridina* also, although they do not seem to incorporate secretory products. It is possible that these, as has been shown by Owen (1955) in the diverticula of *Cardium edule* might serve to convey indigestible material out.

Duct of the digestive divertucila

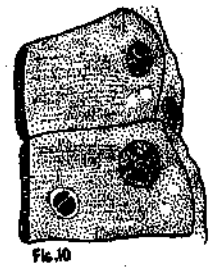
The epithelium of the main duct measures about 18-20 μ in thickness and the lumen about 90μ. The cells are columnar and have large spherical or elongated nuclei with usually two nucleoli. A few cells appear as binucleate. Histologically the duct resembles the tubule of the diverticula. The outer connective tissue envelope of the tubule is continuous with that of the secondary and main ducts.

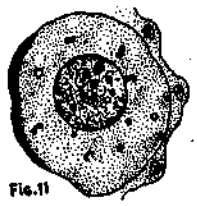
2. The Midgut

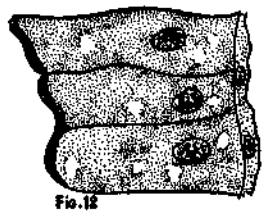
The chitinous lining of the foregut terminates in the region of the pyloric stomach, immediately in front of the origin of the midgut caeca (*vide infra*). The midgut is long and extends upto the last segment. It is strange that the posterior valves of the stomach in *Caridina brachydactyla* (Patwardhan 1935) extend into the hindgut in which case the midgut should be very short.

Histology (Fig. 13)

Enveloped by an outer thin connective tissue layer (ct) are two layers of closely arranged muscle fibres, the longitudinal and circular. Beneath this is a wavy basal membrane and then the epithelial cells (e) of the midgut. The nuclei of the midgut epithelial cells are elongated or oval with usually two nucleoli. Free ends of cells exhibit fine striations. Scattered among the epithelial cells are the spherical compact basal cells (b) (Frenzel 1885) which have been observed in various stages of amitosis. The midgut is divisible into three zones. An anterior zone behind the digestive diverticula distinguishable by the short uneven epithelial cells which are only very faintly stainable. The middle zone has columnar, deeply stainable cells







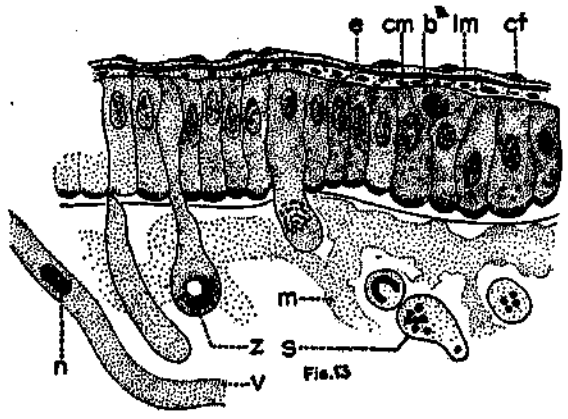


Fig. 10. Dark cell (Modified Champy's fluid).
Fig. 11. Dark cell (Heidenhain's Azan).
Fig. 12. Light cells.
Fig. 13. L.S. through wall of midgut.

measuring about 25μ by 3μ. The posterior zone is constituted by less tall (15μ) but stouter (5μ) cells arranged uniformly. These stain, less intensely than the preceding zone.

Though the epithelial layer of cells of the midgut appear as homomorphous, they show a difference in functional physiology. This is evident on studying the epithelium of the empty midgut in which secretory and non-secretory cells are seen. Numerous spherical or oblong cytoplasmic bodies (s) measuring from 4μ to 20μ are noticeable inside the lumen in all the three zones of the midgut (Plate : Fig. 1).

These are enucleated and contain one or more vacuoles of varying sizes and sometimes a few granule-like concretions. The contents of the majority of the vacuoles at this stage stain red and a few orange in Heidenhain's Azan. In others, part of it alone stains appearing as crescentic or ring-shaped figures. Examination of numerous midgut sections has made it possible to state that these have been formed from some of the midgut epithelial cells. Primarily such epithelial cells show an aggregation of granules, stainable red in Azan, towards their zymogen region (z). The zymogen region is later nipped off into the lumen of the midgut. The granules which apparently represent the secretory products, organise to form vacuoles preceding or succeeding the separation of the zymogen region. The bifold stainability may be explained as due to a slight variation in the chemical composition of the vacuolar contents in its various phases of formation which consequently demonstrates its constitutional affinity, either to azocarmine or orange G. In the midgut of *Glossina* (Wigglesworth 1929) and in the dragon fly nymph (Needham 1898), cytoplasmic globules of a similar nature have been observed to be discharged after feeding.

Lying loose inside the two posterior zones of the intestine are elongated ' vermiform bodies ' (Plate : Fig. 2). These (Fig. 13, v) have a very fine granular cytoplasm which stains a light bluish-red or faint pink. Some of the largest of these measure 80μ by 5μ. Their body is homogeneous without any vacuolisation. In most of these an elongated nucleus-like body (n) about 4μ in length is noticeable. In many of these vermiform bodies, a disintegrating ' nucleus ' with a well-defined 'nucleolus' (1μ across) is clearly observable and in others only the ' nucleolus'. Faint connections between the vermiform bodies and the epithelial layer have been noticed in certain regions of the midgut. At present, it can only be stated that these vermiform bodies are formed by the transformation of a part or whole of some of the epithelial cells accompanied probably by nucleolar extrusion, mostly by disintegration of the rest of the nucleus. Later these bodies spread out to form a matrix inside the lumen.

As far as is known, in none of the Crustacea, reference to such structures has been made. Howells (1942) has described the mucous cells in the salivary glands and in the intestine of *Aplysia punctata.* The mucous cells have been observed to extend into the lumen of the intestine as elongated structures and discharge mucus in masses. Though not drawing a close parallel, it may be stated that the extension of the mucous cells bears a resemblance with the vermiform bodies seen in the midgut of *Caridina.* The spherules of cytoplasm containing either vacuoles or granules referred to before are also found inside the intestine of *Aplysia* (by the same author) in which a few of the gland cells with secretory granules at their tip extrude these into the lumen where the granules swell into thin-walled vesicles. These granules also stain bright red in Azan and the vesicles are supposed to bind the loose intestinal contents into solid faeces.

Animals with empty intestine were tested for mucus using Lillie's method (Cowdry 1952). The result was positive to the matrix and the vermiform bodies. This was confirmed by Mayer's mucicarmine (Cowdry 1952). The mucus from the vermiform bodies binds the faecal matter and this is why the cylinder of extruded faeces, even when divested of its peritrophic membrane, retains its shape in water for a long time.

Following the discharge of the secretory contents, the epithelial cells enter into a period of rest and exhibit signs of atrophy of the nuclei. Later when the intestine

is rendered empty due to continued defaecations, the atrophying cells are seen to be detached into the lumen of the midgut. In some cases very heavy sloughing of the cells has been observed giving rise to masses of cells inside the middle zone of the intestine. This delamination of the midgut epithelium suggests cylic secretory activity and it is assumed that the secretion of the midgut epithelium is holocrine unlike the hepatopancreatic cells.

The peritrophic membrane investing the faecal pellets is extremely thin and transparent. Its property of solubility in concentrated acids and insolubility in concentrated alkalies conforms to that of chitin. The chitosan-idoine colour reaction gives a faint violet colour showing that in constitution, it is similar to chitin. This is in order with the findings of Forster (1953) in some carideans.

Midgut caeca

Midgut caeca consist of three blind diverticula on either side. The middle one is longer and the other two smaller ones are attached to the base of the former. The middle caecum has a small outgrowth just behind its apex. The maximal length of the caeca is 0.65 mm. The two caeca on either side open into the anterior dorso-lateral part of the midgut. Histologically the cells of the midgut caecum resemble closely those of the midgut.

3. The Hindgut

The hindgut commences from the hind end of the midgut in the middle part of the last abdominal segment and is about 0.85 mm. long in an adult female. Separating the midgut epithelium from the hindgut epithelium is a narrow gap where the basal membrane is exposed (Plate : Fig. 3). The junction is devoid of a caecum. The hindgut takes a gradual slope and opens out by the anus on the ventral side of the base of the telson. At its commencement the lumen of the hindgut is narrowed by the development of a thick circular pad (sp) formed by the elevation of the epithelial cells with a core of circular and radial muscle fibres inside. The disposition of the muscles clearly points to this structure as a sphincter which controls the entry of the contents of the midgut into the hindgut. It is 80μ thick and 22μ high. The hindgut is lined by a layer of cuticle which at the hind margin of the sphincter bears a few claw-shaped spinules (s). Rectal valve of a similar nature does not seem to have been described in any Crustacea. In *Calliphora* (Insecta) Graham-Smith (1934) has observed a similar valve with backwardly directed spines. The spines seize the peritrophic membrane and pass it backwards by a series of movements. In *Caridina*, the function of the spinules is not clear although it is probable that they seize the peritrophic membrane and arrest its progression which helps in the concentration of faecal matter before it is expelled. Behind the sphincter are six longitudinal ridges of the rectal epithelium, the rectal pads. Two are dorsal, two lateral and two ventral. The dorsal ones are enormously developed compared to the others, extending deep into the lumen.

Histology of the hindgut

The connective tissue envelope and the outer longitudinal muscle are extremely reduced. The circular layer of muscles assumes better proportions. Restricted to the core of the rectal pads are bundles of longitudinal muscle fibres embedded in connective tissue. These bundles extend fan-wise inside the sphincter so that the

valve and the rest of the hindgut function as a single unit. The epithelium of the hindgut is less inclined to be columnar than that of the midgut. Radial muscles connecting the inner cuticular lining of the rectum with the exoskeleton at its hind end function as dilator muscles.

The anus opens at the summit of a small papilla situated in between the bases of the uropods on the anteroventral part of the telson. It is a longitudinal slit with a pair of tumid lips. The faecal pellets are passed backwards below the telson which presents a ventral longitudinal excavation.

Anal Intakes of Water and Defaecation

METHODS

Being transparent, *Caridina* permits direct observation on the anal intakes of water. Animals collected from the aquarium were observed under the stereoscopic binocular microscope in transmitted light. They were either kept in a small quantity of water or were fastened to glass slides gently by rubber bands to facilitate continuous observation. The count of anal intakes of water was made with the help of a counter. Animals kept in a suspension of fine charcoal were seen to take in particles of carbon during rectal swallowing.

The number of anal intakes of two specimens A and B are given below in the table.

TABLE

ANIMAL A

Number of anal gulps per minute

8	7	7	8	7	8	8	9	9	9	11	9	11*
19	18	14	12	11	10	9	8	9	7	12	11	
11	11	11	11	10	11	12	10	11	11	11		
10	12	12	12	11	12	12	12	11	12	13		
12	11	10	12	11	11	11	11	12	11	12		
11	12	12	12	11	10	11	13	13	12	12		
12	12	12	13	12	12	12	11	12	11	11		
12	11	11	12	11	11	11	11	*	18	18		
14	13	12	11	9	11	9	10	11	12			

ANIMAL B

Number of anal gulps per minute

20	22	22	20	23	*	35	*	37	*	32	*
34	30	30	21	20	23	23	22	*	30	*	
42	*	39	*	37							

(Each asterisk represents one defaecation)

In animal B, the number of intakes immediately after defaecation was calculated for one minute, because successive defaecations took place within fractions of a minute.

When the midgut is full, the middle zone is narrower than the anterior or posterior zones. The wall of the midgut exhibits antiperistaltic waves. There is regular anal intake of water. The two lips of the anus move out engulfing a minute spindle of water which is sent forwards in between the two conspicuous dorsal pads of the rectum. Prior to the process of defaecation the number of anal intakes becomes constant in a particular animal (animal A). But this frequency is seen to vary with different animals (animal B).

During defaecation, the rectum is dilated and the increased pressure inside the intestine drives the faecal matter into the rectum as far as the anus which is kept closed. The sphincter with its circlet of spinules now contracts serving a faecal pellet from the cylinder which is discharged to the outside. A rotary motion of the sphincter with the spinules would well serve the purpose of shearing the peritrophic membrane. The faecal pellets measure about 0.8 to 1 mm. which is also the length of the rectum.

Following defaecation there is a sudden increase in the frequency of the rectal swallowing. The quantity of water ingested in each intake also increases. When the interval between successive processes of defaecation is short, as in specimen B, this accelerated rate of intake is maintained. When the next defaecation is delayed as in specimen A (more than an hour and a half), this mode tones down. The time required for rendering the intestine completely empty varies a good deal depending on the size and activity of the animal.

Even after a few defaecations, the intestine is turgid having a uniform bulge, the spaces between the columns of faecal matter being filled with water. Here the antiperistaltic movements are at their best with the highest amplitude for the waves. Even after rendering the intestine empty, the spells of anal intake continue for a time after which the fluid is discharged to the outside and the intestine becomes flaccid.

It is obvious that the intake of water bears a relationship to defaecation and it seems natural to assume that the intake of water is primarily to maintain a constant pressure inside the gut. The maintenance of an increased pressure could be demonstrated by inserting the fine point of a needle through the anal aparture into the midgut when the intestine collapses to a certain extent discharging part of the fluid content to the outside. Fox (1952) has disproved the respiratory function attributed earlier to the anal intake of water. He is of opinion that it is of the nature of a natural enema which would mean an increased pressure that stretches the wall of the gut until it contracts.

The oral intake of water could not be studied due to the opaqueness of the cephalothorax. It also shows a rhythm with an average of 14 gulps per minute.

Summary

The anatomy and histology of the foregut, midgut, hindgut, hepatopancreas and midgut caeca have been studied. The stomach exhibits a certain complexity of organisation in comparison with other Caridea. The cardiac and dorsal section of the pyloric stomach show a few important resemblances with *Cerataspis*, a penaeid. The absence of a gastric mill has made the extrinisic muscles of the stomach less

developed while the intrinsic muscles are not affected. The working of the stomach and course of food have been studied through the transparent cuticle. The study of the process of secretion and restitution of the gland cells of the digestive diverticulum shows that with the initiation of feeding, the extrusion phase sets in, which lasts for two hours and a half. The restitution phase following this reaches its mode between two hours forty minutes and four hours fifteen minutes. The second extrusion phase is attained five hours after feeding and three hours later the second restitution phase sets in. Certain spherules of cytoplasm and 'vermiform bodies' have been observed inside the midgut and their probable function is discussed. A rectal valve with spinules is described. The frequency, mechanism and probable function of the anal swallowing of water is described.

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Fig. 1. Cytoplasmic spherules containing secretory products.
Fig. 2. L.S. through midgut showing ' Vermiform Bodies '.
Fig. 3. L.S. through initial part of hindgut.