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Anatomical and Histological Structure of Digestive Tract of Adult *Platynotus belli. F* (Coleoptera: Tenebrionidae)

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ABSTRACT: The digestive tract of adult *Platynotus belli.* is anatomically divided into short foregut, long midgut and hindgut which opens outside through anus. Histologically foregut and hindgut showed outer circular and inner longitudinal muscle layers followed by basement membrane and epithelium. Inner most layer is intima. Midgut consists of outer muscularis and inner columnar epithelium with peritrophic membrane surrounding gut content. Malphigian tubule shows single layer of cuboidal epithelium. Crop and salivary glands are absent in this beetle.

Key words: Digestive tract, Adult *Platynotus belli.*

INTRODUCTION

The organs and process of digestion in insects shows great variations due to the variation in consumption of food materials. The successful adaptation of an insect to a particular food as its source of nutrients, essential for growth, development, reproduction and population maintenance & requires a unique combination of behavioral, physiological and biochemical processes (Slansky, 1982). The gut length is generally correlated with diet. Generally the insects which consume higher protein containing diet have shorter gut (Pradhan, 1939). The morphology and histology of alimentary canal of different groups of coleopteran is a matter of interest of a number of authors including Talbot (1928), Miller (1961), Mukherji and Singh (1973).

In Coleopterans generally short straight and tubular foregut is present and can be devided in to anterior short pharynx, and a narrow tubular oesophagous. At the joining of foregut and midgut proventriculus may be present (Ekis and Gupta, 1971) with well developed armature. In coleopterans it function as a grinding organ or as a valve. In many insects it forms a storage organ called crop. The crop is usually absent or very slightly developed in beetle larvae and polyphagous adults, where as usually present in adult Adephaga (Crowson, 1981). The crop may also serve as a site for preliminary or more complete digestion.

Midgut is the largest and longest diversion of the alimentary canal. As midgut is endodermal in origin hence shows absence of chitinous lining. It is well established that the midgut of insects secretes most of the digestive enzymes and is the principal site of digestion (Gilmour, 1961; Dadd, 1970; Wigglesworth, 1972).

In coleoptera midgut is long and coiled in adults like *Trypodendron lineatum* (Schneider and Rudinsky, 1969), *Cephalodesmis armiger* (Lopez Guerrero, 2002). In some species of *Meloidae* the midgut is morphologically differentiated into broad anterior and narrow posterior regions. In some species of *Cleridae* is divided in to anterior, middle and posterior regions based on differences in size and shape also midgut is covered by small papillae, which are the outward projections of crypts. The papillae contain regenerative cells (Chapman, 1998).

Histologically midgut in insects shows three types of epithelial cells as described by Shinoda (1930) which includes columnar, goblet and regenerative cells showing variation in functions in various insects. (Lewis, 1926; Waterhouse, 1952 and Wigglesworth, 1965). Various authors have reported the presence of goblet cells in the Lepidopteron and Coleopteran larvae and described their fine structure and functions (Chapman, 1985; Santos *et al.,* 1984). The presence of goblet cells has also been reported in the midgut epithelium of Ephemeroptera, Plecoptera and Trichoptera (Chapman, 1972).

Hindgut, the last part of digestive system initiates with ileum followed by colon and rectum and ends exteriorly with anus. The hindgut is lined by thin layer of permeable cuticle which avoids loss of useful substance (Maddrell and Gandiner, 1980). Histomorphological details of alimentary canal have been investigated in several species of coleopterous insects (BalfourBrowne, 1934, 1935; Jones, 1940; Swingle, 1950; Gupta, 1965; Berberet and Helms, 1972; Kumar and Adjei, 1975).

Malphigian tubules are the excretory organs in insects at the junction of midgut and hindgut.

The malphigian tubules become closely associated with the rectal pads so that the two water absorbing systems are placed in series. This system is seen in Coleopterans and in most larval lepidoptera and in some larval symphata. Palm (1949) gives a general survey of the rectal pads or rectal glands in different orders of insects. In *Thysanura, Odonata, Orthoptera* and *Phasmida*. Malphigian tubules are composed of single layer of cells and show two layers in *Neuroptera, Hymenoptera, Lepidoptera* and *Diptera.* However, members of largest order Coleoptera are relatively neglected from such studies. Only scanty information is available on the anatomy and digestive histology of some Coleopterans. Earlier literature indicates, there is no information on the *P.belli.* the species under study.

Platynotus belli is a ground beetle which feeds on various types of foods and occasionally becomes pest on flour and stored grains. A number of extensive work have been published on stored product beetles, the more important of them include those of Hinton (1948), Lepesme (1944) and Aitken (1975). The information available indicates that the studies pertaining to histomorphology of digestive tract and digestive enzymes in the flour beetles is scanty. Therefore, to overcome the lacunae, the efforts were made to study histomorphology of alimentary canal of *P.belli.* in order to provide basic knowledge about digestive enzymes and the system this can be useful for controlling the pest.

MATERIAL AND METHODS

A. Collection and maintenance of experimental animal

The experimental animal *Platynotus belli*. F for the present investigation was collected from their natural habitat from Kolhapur city, Maharashtra, India. They were reared and maintained under laboratory conditions throughout the investigation period (2006-2007). This is to make sure that the sufficient enzyme source for carrying out various aspects of enzymes.

Adults were maintained in the earthen pots containing loose moist mixture of sand and soil in equal parts as per the method proposed by Blume and Aga (1975). Maximum 50 beetles were reared successfully in each pot. Lowest layer of pot was provided with medium size stones in order to provide crevices for the beetles to facilitate their burrowing habit. Wheat flour was provided as a food for the beetles during rearing. Water was given with the help of wet cotton balls. This method was found to be suitable for feeding and maintenance of adults. The adults were utilized for histomorphological and biochemical studies.

B. Tissue preparation for histological studies

The adults of *Platynotus belli.F* were dissected in chilled insect ringer solution under stereoscopic dissecting binocular microscope. The alimentary canals were removed and for their gross anatomical structures, measurements were taken. Then various parts of alimentary canal were fixed in different fixatives for 24 hours. The tissues which were fixed in Bouins fixative after 24 hours were washed under running tap water for 12 hours to remove excess fixative and then gradual dehydration of tissue was carried out using ethyl alcohol. The tissues which were fixed in steives fixative were washed in 50% alcohol and transferred to 70% alcohol containing enough iodine for 5 to 8 hours. This step was followed by gradual dehydration as usual. After dehydration the tissues were cleared in xylene, infiltrated and embedded in paraffin wax (52^0C – 54^0C). The tissues were sectioned at 5 to 7 µm thickness. The sections were stained with haematoxylene – Eosin (Delafield) method. After staining observations were made and microphotography was done.

RESULTS AND DISCUSSION

Anatomy: Alimentary canal of *P.belli* showed the characterisitics of Coleopteran. It is long simple, tube with varying diameter and runs along mouth to anus passing from head, thorax and abdomen. It is connected with the body wall in head region by muscles, elsewhere supported by the tracheal branches. The main structures of the alimentary canal are easily distinguishable and it is divided into foregut or stomodaeum, midgut or mesenteron and hindgut or proctodaeum. The length of alimentary canal of is 2 – 2.5 folds that of body length as it measures about 58 – 62 mm long where as length of whole body is 25 – 30mm long. The morphometry of alimentary canal is given in Table 1.

Table 1: Biometry of alimentary canal in adult *Platynotus belli.*

Sr. No.	Parameter	Length (mm)
1.	Total length of body	25-30
2.	Length of fore gut	2
3.	Length of mid gut	34
4.	Length of hind gut	26
5.	Total length of alimentary canal	62
6.	Relation between body size and total length of alimentary canal	2.06

Foregut: Foregut occupies 3.3% of the total length of alimentary canal. It is very short & it almost confined to the head capsule. It consist three regions, preoral cavity, pharynx and oesophagous. The buccal cavity is a very short space just for the conduction of food to the pharynx. It is lined by a thick layer of intima with a brush of stout hair i.e. spiny intima. The pharynx & oesophagous together constitutes a uniform tube measures about half mm in diameter. Crop is absent in this beetle. Posterior most part of the foregut is proventriculus which gradually increases half mm in diameter and further extends in to lumen of midgut.

Midgut: Midgut / Mesenteron is the longest part of alimentary canal extending from the anterior region of prothorax to the seventh abdominal segment, where it forms a loop and combines with the hindgut. Midgut is the main site of secretion, digestion and absorption of the nutrients. It measures about 34mm in length. Diameter of midgut decreases as it tapers towards hindgut. It is about 2mm in diameter which varies in accordance with the quantity of food it contains.The junction between midgut and hindgut is demarcated by origin of malphigian tubules.

Hindgut: Hindgut is divided in to anterior intestine and posterior intestine. These parts are thrown in to two coils. The anterior intestine is 1mm in diameter and it is narrower than the mesenteron and consists of coiled ileum and colon. The posterior intestine or rectum shows straight tubular structure and it is narrower than the anterior intestine. Hindgut comprises 26mm in length. Malphigian tubules are present in this region showing cryptonephric arrangement. Rectum further proceeds in to a narrow anal canal, about half mm in diameter and 4mm in length, which externally opens into an anus.There are no salivary glands observed in *P. belli.*

Anal canal: The epithelium is very much folded and is made up of small cuboidal cells with round nuclei. It is supported by a basement membrane. It bears thick intima internally and very well developed muscle layer externally. The musculature includes inner, longitudinal muscles at the base of folds and outer thick coat of circular muscles

Malphigian tubules: There are in all six malphigian tubules divided in to three groups, each group containing two of them.

Histology:

Foregut: Histologically structure of foregut shows the typical coleopteran pattern. It is externally surrounded by muscularis made up of few strands of outer circular and inner longitudinal muscle layers followed by basement membrane and epithelium. Circular muscle layer is thicker than longitudinal muscle layers. Epithelium is thrown in to six longitudinal folds. It consists of single layer of flattened epithelial cells with indistinct basement membrane. The inner most layer is intima which is thick and chitinous showing folds along with the epithelial layer. Intima is non – cellular layer which lines entire foregut forming longitudinal folds with the epithelial layer. The intima of anterior foregut shows well developed spiny processes.

Cardiac valve: The cardiac or stomodaeal valve is an everted portion of the posterior end of the stomodaeal wall, projecting in to the ventriculus or mesenteron. The epithelium consists of tall columnar cells with oval nuclei. It is lined by smooth intima without any special structure like proventricular teeth, pad or spines. The junction between foregut and midgut is marked by dilation of the midgut due to shallow invagination of the cardiac valve.

Midgut: Histologically structure of midgut shows the following tissues to be much same in size and structure throughout. *i.e.* epithelium of endoderm cells supported by a basement membrane, inner circular muscles and outer longitudinal muscles.

Fig.1

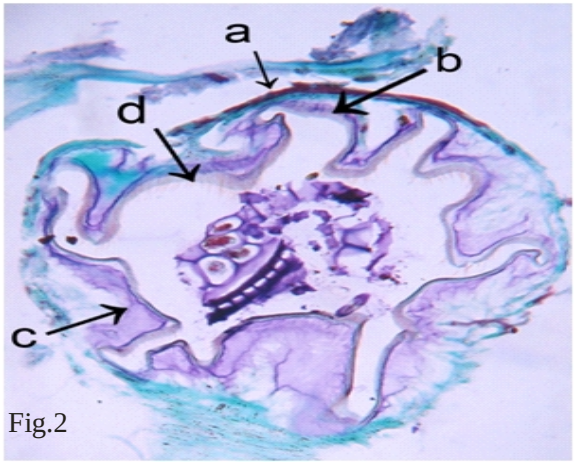

Fig.2

Fig.3

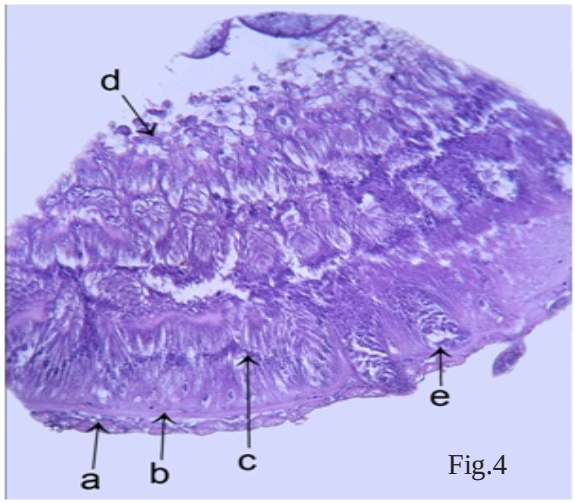

Fig.4

Fig. 1. Whole mount of alimentary canal of *Platynotus belli* adult.a - Pharynx; b - Oesophagous; c - Midgut; d - Ileum;e – Colon; f- rectum; g - anal canal; h - anus. **Fig. 2 T. S. of pharynx** showing a – Circular muscles; b – longitudinal muscles; c – epithelium; d – intima with spines. (40X) **Fig. 3 T.S. of oesophagous** showing a – Circular muscles; b – longitudinal muscles;c – folded epithelium; d – intima. (10X) **Fig. 4 T.S of midgut** (Magnified) showing a – circular muscles; b – thin layer of circular muscles c – well developed columnar epithelial cells; d – peritrophic membrane e – gut content. (40X).

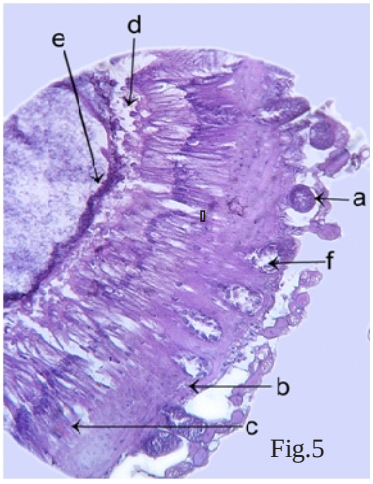

Fig.5

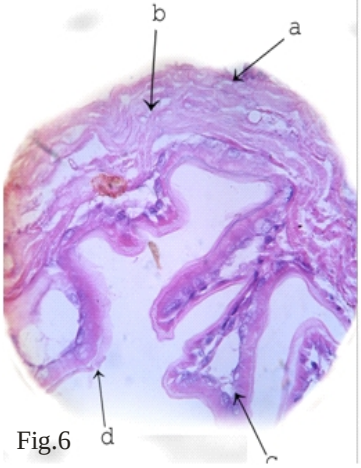

Fig.6

Fig.7

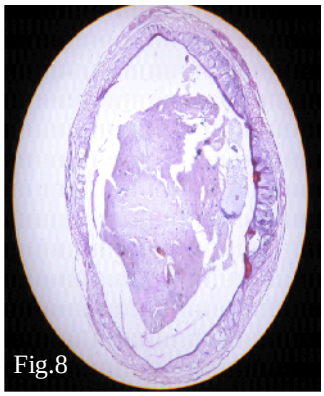
Fig.8

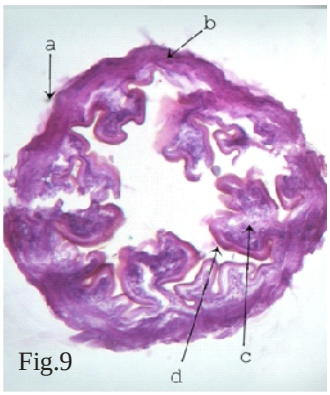

Fig.9

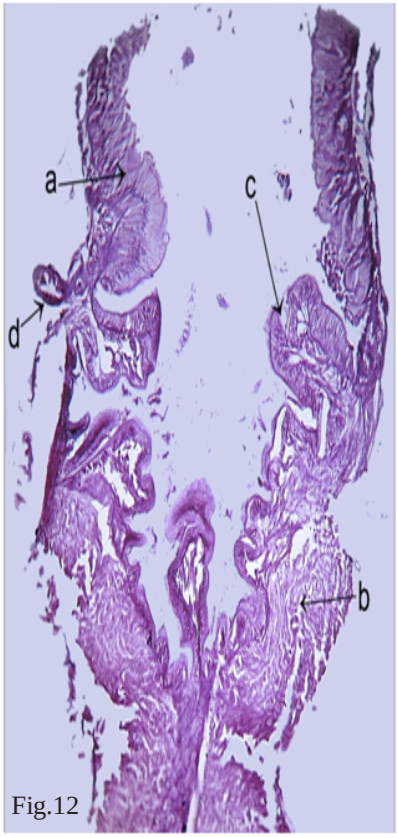

Fig.12

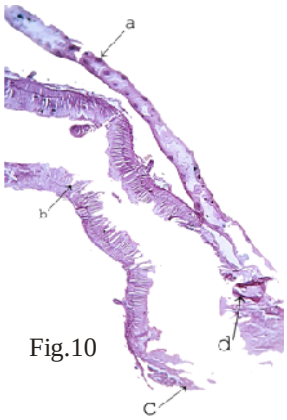

Fig.10

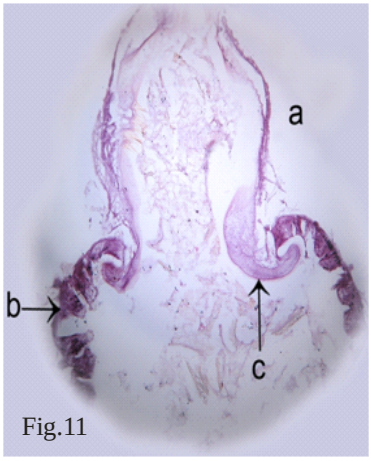

Fig.11

Fig. 5. T.S of midgut (Magnified) showing a – circular muscles; b – thin layer of circular muscles c – well developed columnar epithelial cells; d – peritrophic membrane e – gut content. (40X). **Fig. 6 Section of Ileum** Note the circular muscles (a); longitudinal muscles (b); Folded epithelium with cuboidal cells (c) and intima (d). (40X) **Fig. 7 T.S of colon** showing a – longidunil muscles; b – thick Circular muscles; c – folded epithelium; d – intima. (10X) **Fig. 8 Section of rectum** Note the – few longitudinal muscles; b – circular muscles; c – cuboidal epithelium; d – intima. (10X) **Fig. 9 T.S. of anal canal** (posterior region) Note the longitudinal muscles (a); circular muscles (b) Folded epithelium (c) & intima (d). (40X)**Fig. 10 L.S passing through Malpighian tubule** and Midgut of adult *P.belli* Note single layered epithelium (a); midgut epithelium (b); hindgut (c) and opening of malpighian tubule in gut (d). (10X) **Fig. 11 L. S. of passing through fore gut-mid gut junction** showing, a-fore gut,, b- mid gut, & c- folds of foregut epithelium with intima projecting in to mid gut lumen forming stomodael valve. (40X). **Fig. 12 L.S passing through midgut – hindgut junction**. a – midgut; b – hindgut; c – folds of midgut epithelium forming pyloric valve. d – malpighian tubule opens at the midgut- hindgut junction and midgut intima. (10X).

The cells of epithelium are tall, columnar with distinct cell membranes and conspicuous, centrally placed nuclei. Epithelium is supported by basement membrane and its apical portion bears striated border. The striated border of the cells disappears in the secretary phase showing holocrine secretion. The regenerative cells are small lie at the bases of functional cells in groups at regular interval and, scattered throughout the length of the ventriculus. Striated circular muscle fibers surround the epithelium.

The ventricular wall gives out numerous small pouch like diverticula, the gastric caecae. They are distributed throughout the wall of the ventriculus. Some of them are completely embedded in the circular muscle layer. In the section they appear as it filled with small darkly stained nuclei. The food content is enclosed in a peritrophic membrane

Hindgut: *Anterior intestine:* Even though not marked externally, histologically the anterior intestine is in the tract forms the demarcating zone of midgut and the anterior intestine divisible in to anterior ileum & colon.
Ileum: Histologically the muscularis includes poorly developed outer longitudinal muscles and inner thick coat of circular muscles. The epithelium of ileum consists of cuboidal cells supported by a prominent basement membrane. The nuclei of the epithelial cells are small & round. The intima is thin, chitinous and is provided with small spines
Colon: Histologically colon bears thick circular and longitudinal muscles covering and is lined internally by thick layer of chitinious intima. The epithelium is made up of cuboidal cells with very large oval prominent nuclei with well defined cell boundaries and internally lined with thick layer of chitinous intima
Posterior intestine: The posterior intestine, rectum is divided in to anterior rectum and posterior rectum. The epithelium of rectum bears broad folds and is made up of large cuboidal cells with large round nuclei. Internally, the epithelium is lined by thick intima and externally by isolated few longitudinal and well developed circular muscles. The longitudinal muscles are thrown into six external lengthwise bands give somewhat hexagonal appearance to the rectum. There are no salivary glands observed in *P.belli.*
Anal canal: The epithelium is very much folded and is made up of small cuboidal cells with round nuclei. It is supported by a basement membrane. It bears thick intima internally and very well developed muscle layer externally.

The musculature includes inner, longitudinal muscles at the base of folds and outer thick coat of circular muscles.

Malphigian tubules: Each malphigian tubule is unbranched tube, having uniform diameter throughout its length. The tubes run forward on the midgut and then turn posterior and forms series of convolution around the hindgut. The section of malphigian tubule show single layer of cuboidal epithelium. The cells contain large nuclei. Externally there is a thin connective sheath. The cells of the proximal region are larger, where as distal region are flattened.

The digestive gut shows resemblance with some of the coleopteran species like *Polyphylla decemlineata, Tribolium confusum, Tenebrio moliter.* (Hafeez and Gardiner 1964; Gerber, 1976). The gut is divided in to foregut, midgut and hindgut. Similarly in *Pseudaletia sequax* (Gongalves, 1981), *Danais archippus* (Burgess, 1880), *Hyalophora cercropia* (Judy & Gilbert, 1970). In other beetles like *Holotrichia serrata, Holotrichia Fissa, Leucopholis lepidophora and Chiloloba orientalis*, the general anatomy of digestive system seems to be similar as in *P.belli*. (Berberet & helms, 1972).

Length of the digestive gut depends on the feeding habit of the insect. Adults of *P.belli* feed on the decaying matter. In culture it was provided with wheat flour. It has been observed that length of alimentary canal is 2.5 times that of body length of *P.belli* where as in other beetles like *H.serrata* reported 3-4 times longer alimentary tract and 5-6 times longer in *L.lepidophora*. In some insect's digestive gut is found to be very long as compared to body length such as eight times longer in *Phanaeus vindex,* and ten times longer in *Canthon pilularis* and *Dichotomius carolinus*. The entire digestive tract is not much longer than the body length in *O. rhinoceros* (Gressitt, 1953).

Foregut in *P.belli* is very short tube which simply acts as a passage for the transfer of food from preoral cavity to the active site of digestion i.e. to the midgut. This is true for all most of the beetles studied so far (Bacton ,1930, Swingle, 1950; Berberet & Helms, 1972; Edmonds 1974) . Crop is absent in the beetle under study as shown in other tenebrionids like *Tribolium anaphe, Tribolium castaneum* and *Tribolium confusum* (Hafeez & Gardiner, 1964).

Midgut is longest part of alimentary canal, comprising 58.33% of total gut length. Diameter of midgut decreases as it tapers towards hindgut showing anterior and posterior midgut regions morphologically.

Ferreira *et, al.* (1981) has reported midgut divided in two subdivisions morphologically in *Rhynchosciara* fly. The hindgut in scarabaeid beetles, may be long or short depending upon the species. In *P.belli* hindgut is last part of alimentary canal divided into anterior and posterior intestine consisting of ileum, colon and rectum. In beetles the larvae and adults consist of anterior intestine, ileum and posterior colon and rectum. (Becton 1930, Swingle, 1950; Berberet and Helms, 1972).

In the present species, salivary glands are absent as in majority of Coleopteran. Similar observations were made by Kumar and Adjei (1975) in *Lucicola discicolis, O. philemon, O. catta, C.arrowi, and L. rhadamistus.*

Histology of foregut of adult *Platynotus belli* is comparable with the histology of other Coleopteran type studied, as it posses the basic plan i.e. well developed muscularis, basement membrane, epithelium thrown in to longitudinal folds with internal intima and formation of cardiac valve by invagination of epithelial folds in the lumen of midgut at foregut – midgut junction. (Berberet and Helms, 1974). The syncitial epithelium in the foregut of *Hister* (Sexena, 1966) and foregut as well as hindgut of *Cybister limbatus* seems to be an artifact due to fixation because of thick intima. The indistinct cell boundaries were also observed in the boll weevil (Chadbourne, 1961). On the contrary, the cell boundaries are very distinct in all parts of alimentary canal of *P.belli*. The intima of pharynx of this species show similar caudally directed spines as in other beetles. (Becton, 1930; Jones, 1940; Swingle, 1950). The oesophageal intima of *P.belli* is devoid of spines.

As far as histology of midgut is concerned, it is a constant feature of Coleopteran ventricules i.e. columnar secretary epithelium with prominent nuclei and striated border which is observed in *P.belli*. The epithelium is thrown in to number of longitudinal folds to ensure the efficient digestion and absorption of food by increasing the cell number and surface area. According to Snodgrass (1935) in many Coleoptera, a large portion of ventriculus is covered with small papilliform or sometimes elongate diverticula, but these structures in most cases are the crypts of epithelial regenerative cells rather true caeca. But in *P.belli* large number of nucleated vesicles is given off in the lumen from these papilliform gastric cacea.

In the present species, on the free border of epithelial cells, secretion of globules was observed indicating holocrine secretary nature. Bhave (1981) in *Dineutus indicus* observed vesicles in the lumen of the caecae, which were filled with secretary matter. Snodgrass (1935) felt that, the subject must be studied from a physiological stand point before these conclusions could be finally accepted. In *P. belli* epithelial cells are replaced by regenerative cells scattered in the general epithelium at regular intervals. The peritrophic membrane was very thin membrane surrounding the gut content of midgut in *P. belli*. It is secreted by cells at the base of reflexed layer of cells of cardiac or oesophageal valve. The peritrophic membrane was first reported by Aubertot (1934) in *Pieris brassicae.* It surrounds the food material separates it from the midgut wall and protects the midgut epithelium from the injury. Such membrane is found many insects (Wigglesworth, 1930; Swingle,1932; Berberet and Helms, 1972). Moreover peritrophic membrane is freely permeable to the digestive enzymes and the products of the digestion.

In *P.belli* hindgut is devided in to two parts, they are anterior intestine / rectum. The epithelium shows longitudinal folds in ileum and colon and is made up of cuboidal cells with prominent nuclei. The epithelium is lined internally by thick intima (Snodgrass, 1930; Swingle, 1930; Jones, 1940; Gressitt, 1953). The muscularis is much more developed in the hindgut than foregut and midgut. The circular muscle layer is very thick layer. However, the outer longitudinal muscles are very few. The rectum is found to be typical insectan type, the section of rectum shows folded epithelium and thicker intima than colon having well developed muscularis. In *P.belli* it is a narrow passage connecting the rectum with anus.

Presence of six malphigian tubules opens laterally into the anterior end of intestine in *P.belli* is similar in those observed by Burgess (1880) and Pyle (1940). It shows single layer of large epithelial cells, bounded externally by basement membrane.

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