Acta Biol. Szeged. 30, pp. 143-152 (1984)

COMPARATIVE MORPHOLOGICAL STUDIES ON THE ALIMENTARY CANAL OF ISCHNURA ELEGANS VANDERL. AND LESTES VIRIDIS VANDERL. (ODONATA, ZYGOPTERA) LARVAE

KATALIN HALASY and MÁRIA CSOKNYA

Attila József University, Department of Zoology, Szeged Janus Pannonius University, Department of Zoology, Pécs

Received June 30, 1983)

Abstract

Macroscopic and microscopic observations concerning the alimentary canal of larvae of two *Zygoptera (Odonata)* species are reported. The histological layers of the trichotomous intestinal canal are muscle, epithelium and intima. The layers vary in thickness in the different sections of the intestine. The indentation observed in the gizzard as well as the regenerative and absorptive epithelial cell types of the midgut are noteworthy.

Key-words: Morphology of the gut in Zygoptera larvae.

Introduction

The majority of the studies on the anatomical, light- and electron microscopic structure of the intestinal canal of insects have dealt with species of health or agricultural importance. The epithelium of the midgut, its morphological and fine structural characteristics have been studied by BROWN (1980) in the tse-tse fly, by HECKER et al., (1971) in the malaria mosquito, by CIOFFI (1979) in *Manduca sexta* — an insect damaging tobacco — and by YU (1981) in the cabbage butterfly. Only few data are available on *Odonata* species, more exactly their larvae. ANDRIES (1970) has reported on the intestinal activity and regeneration of the *Anisoptera Aeschna cyanea* during the digestive cycle and MOENS (1980) on the hindgut of larvea of a few *Anisoptera* species.

In the course of examinations regarding the respiration and osmoregulation of dragonfly larvae it became justified to obtain exact knowledge of the distinct parts and structure of their digestive mechanism since the mentioned functions are in relationship with certain sections of the digestive system (BODINE, 1918; CAROLL, 1918; CIOFFI, 1979; KOCIAM, 1930; KOMNICK, 1977; LEE, 1929; MOENS, 1980; TIL-LYARD, 1917). As our previous studies were carried out on Zygoptera species we chose *Ischnura elegans* VANDERL. and *Lestes viridis* VANDERL. for these investigations too.

Material and methods

Specimens in the last larval stage, approximately equal to size, 21–22 mm in length including the caudal branchiae were examined. The larvae were collected from small deadwaters near Szeged in May, 1981. For light microscopic histology the material was fixed in Bouin's and Carnoy's fixative and in 10% neutral formalin then embedded in paraffin. 5–7 μ m sections were stained with haemateineosine or according to Mallory.

KATALIN HALASY AND MÁRIA CSOKNYA

For electron microscopy the material was fixed in Karnovsky's fixative for 4 h at +4 °C then treated at room temperature with 2% osmium tetroxide (pH: 7,2) dissolved in Millonig's buffer. After dehydration in an ascending alcohol series the material was contrasted overnight with uranyl acetate dissolved in 70% ethanol and it was embedded in Durcupan ACM synthetic resin. The ultrathin sections were prepared with Reichert ultramicrotome and recontrasted with lead citrate. The sections were examined and photographs were taken in a JEOL 100B electron microscope.

Of the numerical data referring to the lenght of the certain parts of the intestine each represents the average of the results measured on 10 larvae being of the same degree of maturity.

Results and discussion

The alimentary canal of the studied Zygoptera larvae is a straight tube extending from the pharynx in the head till the anus in the last abdominal segment; it doesn't penetrate the caudal gills. All parts of the intestine characteristic of the Arthropods in general are to be found in dragonfly larvae (ARA, 1976; BEHURA et al., 1974; MACGOWN et al., 1981; MAKHDOOMI, 1977; QUARTEY et al. 1973).

The stomodeum is the intestinal section reaching from the head to the 2nd or 3rd abdominal segment. It is gradually dilating backwards and suddenly narrowing by its end. It consists of the pharynx, the oesophagus, the ingluvies and the gizzard



stomodeum

mesenteron

proctodeum

Table 1. Tripartition of the alimentary canal of the Zygoptera larvae. Table 2.:

- Plate 1. Longitudinal section of *Lestes viridis* foregut with longitudinal plication. ph=pharynx; oe=oesophagus; Ms=entry of gizzard; m=muscle; ep=epithelium; i=intima l=lumen. Haematein-eosin stain. x 150
- Plate 2. Longitudinal section of *Ischunra elegans* grinding stomach (gizzard). The entry of the stomach can be seen in the upper part of the picture and below the valves terminating towards the midgut (b). On the insert the arrow shows the grinding indentation. m=muscle; ep=epithelium; i=intima; l=lumen. Haematein-eosin stain. x 150 (insert: x 200)
- Plate 3. Longitudinal section of the midgut wall of *Lestes viridis* with dark and light cells. m=muscle; ep=epithelium; i=intima; l=lumen; Haematein-eosin stain. x 200.
- Plate 4. In the cross-section picture of the *Ischnura elegans* hindgut the epithelium (ep) forms 6 longitudinal plicae. m=muscle; i=intima; Mt=Malpighian tubules. Haematein-eosin stain. x 200.

Table 2.



(Table 1). This applies both species studied by us and the corresponding parts of the intestine appear identical in shape and size. The pharynx, about 2 mm long, is a strongly dilating tube situated in its whole length in the head. From there it suddenly widens to the double or triple and continues in a section of spindle form (length, 3,5—4 mm) along the thorax. Even when filled with aliment a longitudinal plication is detectable (Table 2, Plate 1.). which is more striking in *Ischnura elegans* than in *Lestes viridis*. The aliment is stored for a considerable period near the end of this intestinal section since, it cannot reach the following section but at determined intervals. Due to this role of storage this part of the oesophagus is called ingluvies (TIL-LYARD, 1917; WHEDON, 1918).

The thickest and strongest part of the foregut the gizzard is in the abdomen (Table 2. Plate 2.) Its size and location somewhat differs in the two species and in *Ischnura elegans* there are differences between the sexes. In the female individuals of *Ischnura elegans* the gizzard being 0,5—0,8 mm long ends in the 2nd abdominal segment, while in the male individuals it is larger ending in the 3rd abdominal segment. There are no sex differences regarding *Lestes viridis*. Similar data have been reported by others: for example in *Calopteryx* species the location of the gizzard differs in the two sexes however in *Lestes unguiculatus* the gizzard is of the same size and location in male and female individuals (TILLYARD, 1917; WHEDON, 1918). Valves and strong plication can be seen at both the inlet and the outlet of the gizzard, particularly well in histological sections (Table 2. Plate 2.).

The mesenteron or midgut is to be found in the abdominal segments 3—6, or 4—6, being a 3—3,5 mm long section uniform in structure. The Malpighian tubes can be observed at its end parts (Table 1).

The anatomical relations of the hindgut are the most controversial. TILLYARD (1917) described the hindgut of various Zygoptera larvae as being uniform in structure while others (BODINE, 1918; CAROLL, 1918; CULLEN, 1918; KOCIAM, 1930; KOMNICK, 1977; LEE, 1929) found different sections in it. It the larvae studied by us the hindgut could be divided into two parts: the frontal ileum being tubular in shape (3 mm), and the vestibule (0,5 mm) ending with the anus.

Histologically the alimentary canal can be divided into 3 layers, such as the outer muscular layer followed by the epithelial layer on the basal lamina and then the innermost intima which is the product of the epithelial cells (Table 2. Plates 1—4.). These layers are found along the whole intestine however they may become modified according to the functions of certain intestinal sections. The greatest variation is shown by the epithelial layer which is built up of cell indviduals along the alimentary canal. In the foregut the cells are flat in the area of the pharynx., oesophagus and ingluvies with a homogenous intima (cuticula), a layer ensuring the protection of epithelial cells (Table 2. Plate 1.) On the base of the cells a structureless thin basal lamina and a similarly thin muscular layer can be distinguished. The separation of the oesophagus and ingluvies seems groundless on the basis of the histological sections. Their structure is alike and they have the same function: storage of nutriments.

The gizzard differs in structure from the other foregut sections (Table 2. Plate 2). The epithelial layer is made up cuboid epithelial cells and the intima covering the surface differs in the two species. In *Ischnura elegans* there is an indentation in the centre of the gizzard arranged in lo layers. In *Lestes viridis* such an indentation was not detected. These teeth — together with the rather thick muscle layer — pre-sumably assist the mincing of the aliment.



Table 3. Absortive epithelial cells of the midgut of *Ischnura elegans*. Apical section with microvilli (Mv). Er=endoplasmic reticulum; M=mitochondria; Mi=myeline figure; db=dense body; Gr=granule. The arrow indicates a mitochondrion of peculiar shape. x 7700.

Valves and extremely developed plicae are to be found at the inlet and outlet of the stomach. The plicae are formed by the oesophagus at the fore-part of the stomach and by the gizzard itself at the end part. The right and the left valve plicae are identical in appearance therefore these seem to regulate the proceeding of a determined amount of aliment, less probably the direction of progress (KAUSHIK, 1969).

The epithelial cells of the midgut vary in staining with haematein-eosin (Table 2. Plate 3.). Some of the cells stain densely and are elongated, cylindrical, the others show paler staining and are flatter. Latter form small groups among the dense cells. The pale so-called regenerative cells are small having large nuclei. They occur in particularly large numbers in the caudal section of the midgut, similarly as in that of the Anisoptera larvae (ANDRIES, 1970). Electron microscopically, the cylindrical epithelial cells of the midgut show the characteristic appearance of absorptive cells (Table 3). There are many elongated microvilli on their apical surface with dense bodies close to their stem. Mitochondria, rough-surfaced endoplasmic reticulum and lipid granules are seen in the cell plasma. The mitochondria are occasionally vacuolized or are of characteristic form resembling telephone receiver (Table 3. arrow). Multivesicular bodies (Table 4. Plate 1.), large (diameters of 100-150 nm without coat, and the double with coat) coated vesicles having particular structure (Table 4. Plate 2). furthermore rather high amounts of various-sized lipid droplets (Table 4. Plate 3.) can be found in the central region of the epithelial cells. Nearing the basal lamina we are aware of light-microscopically distinguishable regenerative cells among the absorptive ones. The regenerative cells have large nuclei rich in chromatin and narrow perinuclear cytoplasmic rim (Table 5).

The fundamental histological layers are also present in the hindgut: the outer muscular layer then the cylindrical epithelial layer on the basal lamina with rather thick cuticle on their surface facing to the lumen. These layers can well be distinguished in both the haematein-eosin stained sections (Table 2. Plate 4) and the electron micrographs (Table 6). At high magnification basal invaginations are seen on the basis of the cells. Rather contradictory data have been reported regarding the function of the hindgut: according to BODINE (1918) and CAROLL (1918) the rectum serves the function of respiration - tough it doesn't form respiratory chamber in these larvae and it functions in similar manner as the tracheal gills. This assumption is doubted by several authors since it is not supported by epithelial cells supplied with tracheas (KROGH, 1941); on the other hand no relationship can be demonstrated between the frequency of the ileal contractions and the oxygen content of the surrounding water (PENNAK et al., 1944). CIOFFI (1979) and KOMNICK (1977) suggest that considering the lack of ion-absorption areas on the body surface of Odonata larvae, osmoregulatory functions are served by this area of the hindgut in Zygoptera larvae. These authors designated the high cylindrical epithelial cells of the ileum as chloride epithelia and they have proved their assumptions experimentally. On the basis of the obtained morphological picture we accept this latter up-to-date viewpoint.

Table 4.:

Plate 1. Multivesicular body (Mb) in the absorptive epithelial cell of *Ischnura* midgut. Gr=lipid granule. x 34,100.

Plate 2. Coated vesicle (Cv) in the absorptive epithelial cell of *Ischnura* midgut. M=Mitochondrion. x 47,500

Plate 3. Medial area of the epithelium cells of the *Ischunra* midgut rich in lipid granules (Gr). Er=endoplasmic reticulum. x 16,400. Table 4.





Table 5. Regenerative cells among absorptive epithelial cells on the basal lamina (Bl). N=nucleus; Bi=basal invaginations. x 9,700.



Table 6. Detail from the hindgut of Ischnura elegans. The thick cuticle (Cu) is of lamellar structure above the cylindrical epithelial cells. N=nucleus; Bi=basal invagination; trb=tracheoblast; Vm=visceral muscle. x 4,000.

References

- ANDRIES, J. C. (1970): Activité des nids de régénération de l'intestin moyen de la larva d'Aeschna cyanea au cours d'un cycle digestif. — J. Insect Physiol. 16. 1961—1973.
- ARA, P. (1976): Gross morphology of the digestive tract of some Cecidomyides. Gecid. Indica 11, 57—67.
- BEHURA, K. B., DASH, M. M. and BEHERA, K. S. (1974): On the morphology and histology of the digestive system of the giant water bug, *Belostoma indicum* Lep. and Serv. (*Belostomidae*, *Heteroptera*). — Prakrnti-Utral Univ. J. Sci. 11, 11–28.
- BODINE, J. H. (1918): Experimental results in Ischnura and Enallagma. Proc. Acad. Nat. Sci. Phila. 70, 103—113.
- BROWN, R. P. (1980): Ultrastructure and function of midgut epithelium in the tsetse Glossina morsitans Westw. (Diptera: Glossinidae). - J. ent. Soc. sth. Afr. 43, 195-214.
- CAROLL, M. (1918): The hindgut, abdominal tracheae and rectal respiration in the larva of Mecistogaster modestus from Costa Rica. — Proc. Acad. Nat. Sci. Phila. 70, 86—103.
- CIOFFI, M. (1979): The morphology and fine structure of the larval midgut of a moth (Manduca sexta) in relation to active ion transport. Tissue and Cell 11, 467—479.
- CULLEN, A. M. (1918): Rectal tracheation of Argia putrida larva. Proc. Acad. Nat. Sci. Phila 70, 75—81.
- HECKER, H., FREYVOGEL, T. A., BRIEGE, H., and STEIGER, R. (1971): Ultrastructural differentation of the midgut epithelium in female Aedes aegypti (L.) (Insecta, Diptera) imagines. — Acta Trop. 28, 80—104.
- HOUK, E. J. (1977): Midgut ultrastructure of *Culex tarsalis (Diptera: Culicidae)* before and after a blood-meal. Tissue and Cell 9, 103—118.
- KAUSHIK, S. C, (1969): On the morphology of the giant water bug, *Belostoma indicum* Lep. and Serv. (*Heteroptera: Belostomatidae*). Part IV. Morphology and histology of digestive system — J. Anim. Morphol. Physiol. 16. 1—16.
- KOCIAM, V. (1930): O respiracnim vyznamu tracheálnich lupénku u Agrionu. Publ. Fac. Sci.Univ. Charles 108, 1—40.
- KOMNICK, H. (1977): Chloride cells and chloride epithelia of aquatic insects. Int. Rev. Cytol. 49, 285—329.
- KROGH, A. (1941): A comparative physiology of respiratory mechanism. Univ. Press, Pennsylvania.
- LEE, M. O. (1929): Respiration in the insects. Quart. Rev. Biol. 4, 213-232.
- MACGOWN, M. W. and SIKOROWSKI, P. P. (1981): Digestive anatomy of the adult boll weevil, Anthonomus grandis grandis Boheman (Coleoptera: Curculionidae). — Ann. Ent. Soc. Am. 74, 117—126.
- MAKHDOOMI, S. M. A. (1977): Comparative study of the alimentary canal in the larvae and adults of Syrphus balteatus Degeer. — J. Agric. Res. 2, 110—121.
- MOENS, J. (1980): Microscopic and ultrastructural study of different epithelial elements in the ileum of larvae of Anisopteran dragonflies. — Odonatol, 8, 309—315.
- PENNAK, R. W. and MCCOLL, C. M. (1944): An experimental study of oxygen absorption in some dragonfly naiads. — J. Cell. comp. physiol. 23, 1—10.
- QUARTEY, S. Q. and KUMAR, R. (1973): Structure of the alimentary canal and reproductive organs of some adult Neuroptera. — Ent. scand. 4, 91—99.
- TILLYARD, R. J. (1917): The biology of the dragonflies. Cambridge Univ. Press Cambridge, England.
- WHEDON, A. D. (1918): The comparative morphology and possible adaptations of the abdomen in the Odonata. — Trans. Amer. Ent. Soc. 44, 373—437.
- YU, C. H., KIM, W. K. and KIM, C. W. (1980): Secretory granular cells in the midgut epithelium of the Blattella germanica L. — Kor. J. Electr. Micr. 10, 27—32.
- YU, C. H. (1981): Morphology of the midgut epithelial cells in the cabbage butterfly *Pieris rapae* L. — Bull. I.B.S. Inha Univ. 2, 93—99.

Adress of the authors: DR. KATALIN HALASY DR. MÁRIA CSOKNYA Department of Zoology A. J. University H-6701 Szeged, P.O. Box 659 Hungary