Acta Phytopath. Entom. Hung. Vol. 22(1–4), pp. 375–382 (1987)

THE DIGESTIVE SYSTEM OF ADULT CARABID BEETLES: AN ULTRASTRUCTURAL AND HISTOENZYMOLOGICAL STUDY

M. F. Jaspar-Versali[x], G. Goffinet and Ch. Jeuniaux

Laboratory of Morphology, Systematics and Animal Ecology,
Zoological Institute, University of Liège, 4020 Liège, Belgium

The morphological organization and the enzymatic equipment diversity of each digestive segment were studied by means of histology, electron microscopy and histoenzymology in seven carabid species: *Carabus splendens*, *C. nemoralis*, *C. arvensis*, *C. problematicus*, *C. cancellatus*, *Abax parallelepipedus* and *Pterostichus melanarius*.

A particular function was attributed to each alimentary canal segment. The foregut is a mechanically functioning unit ensuring filtration and, in some cases, the site of temporary food storage. It is also the main site of food digestion, the digestive enzymes originating from regurgitated midgut fluid. The anterior region of the midgut plays a role in secretion and absorption processes, whereas the posterior one is the site of intracellular products storage, as well as of excretion and hydromineral regulation. The gastric caeca have a double function since they contain nests of regeneration cells and digestive glands. The hindgut displays ultrastructural features that can generally be related to ionic regulation.

The fact that the digestive enzymatic equipment of carabids is well diversified (proteases, several polysaccharidases and oligosaccharidases) can be interpreted as a primitive character.

In spite of the numerous works dealing with carabid beetles (see review in Thiele 1977), little attention was paid to their digestive system. On the other hand, morphological criteria (Jeannel 1941) as well as some biochemical data (Jeuniaux 1971) support the view that carabids constitute a primitive group among the Coleoptera. It was thus interesting to determine whether the structure and the physiological capabilities of the carabid

---

[x]Senior Research Assistant of the National Fund for Scientific Research (F.N.R.S.) (Belgium)

Akadémiai Kiadó, Budapest

digestive system are primitive ones when considered in an evolutionary context. In this respect, the present paper aims to describe the fine organization of the digestive system of seven carabid species on the basis of histological and electron microscopical observations. For each of them, it attempts also to specify the enzymatic spectrum at the different digestive segment levels by means of histoenzymological methods.

MATERIALS AND METHODS

The following carabid species were investigated: Carabus splendens Oliver, C. nemoralis Müll., C. arvensis Hbst, C. problematicus Hbst, C. cancellatus Ill., Abax parallelepipedus Pill. and Mitt., and Pterostichus melanarius Ill.

Morphological observations were undertaken by means of histology, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Enzymatic activities were detected and localized by histoenzymology according to the methods of Arnould and Bouchez-Decloux (1978) and Jaspar-Versali et al. (1982) for amylase, cellulase, chitinase and laminarinase, of Michel and Chretien (1979) for proteases, of Rutenburg et al. (1960), Hayashi (1965) and Lojda (1972) for oligosaccharidases. The results were expressed in terms of histoenzymological units as previously defined by Jaspar-Versali (1985 and unpublished).

RESULTS AND DISCUSSION

1. Foregut

The carabid beetle foregut is characterized by octogonal symmetry and specialized cuticular features. On the basis of SEM observations concerning general organization, muscle disposition, cuticular thickness, organization and orientation of specialized cuticular features, we can define the structure and the role of the foregut (Jaspar-Versali et al. 1985 a, b). As an example, there are regularly disposed teeth in the oesophagus and a complex architecture of plumose setae in the proventriculus.

Thus the foregut is a mechanically functioning unit and the proventriculus can be considered as a valvular organ, ensuring filtration and, in some species, food storage. Concerning the hypothesis of a triturating function, frequently attributed to the proventriculus (Balfour-Browne 1944, Skuhravy 1959, Zhavoronkova 1969, Cheeseman and Pritchard 1984b), our observations do not support it, at least in the species studied here. The plumose setae found cannot contribute to any food trituration, especially when the food items contain fragments of arthropod cuticle.

The results of TEM study confirm the mechanical function attributed to the foregut. Teeth and plumose setae are epicuticular tanned structures connected to an elaborate system of epicuticular roots sunken into the first procuticular lamellae. This "root system" thus ensures a strong anchorage of teeth and setae in the procuticle. Such roots were also described in other cuticular structures subjected to frequent deformations, such as articular membranes of Carcinus maenas (Compere and Goffinet 1987).

Moreover we observed the development of a muscle attachment system, the organization of which is fundamentally similar to the one described by Caveney (1969) in the integument of arthropods. This system ensures the transmission of muscle contractions to the skeleton and therefore the cohesion and the interdependence of the whole "muscular fibre-epithelium-cuticle" complex.

Our ultrastructural observations also lead to the idea that stomodeal cuticle is regularly rehandled due to wear and abrasion of the external cuticular belts. Thus the intra- and epicuticular canals observed at this level are likely to serve as transport ways for secretion products towards the cuticular upper layers.

All these structural features can be related to the deformations and mechanical tensions exerted on the foregut when a food bolus passes through.

The foregut of carabids, as in other insects (see review in House 1974), is also the main site of food digestion. This hypothesis was first proposed by Schlottke (1937). Our histoenzymological observations revealed that digestive enzymes, such as chitinase, cellulase, amylase, laminarinase and proteases, were present in the stomodeal lumen but never originated from the epithelium (Table 1). Oligosaccharidases only were detected in the foregut epithelium. As carabid beetles are devoid of salivary glands (Wigglesworth 1972), we proposed (as Spence and Sutcliffe 1982, in the case of Nebria brevicollis F., and Cheeseman and Pritchard 1984a, in the case of Scaphinotus marginatus) that the digestive enzymes in the foregut originate from regurgitated midgut fluid.

2. Midgut

From a morphological point of view, the midgut shows two distinct regions: the midgut *sensu stricto* and the gastric caeca covering this organ.

The gastric caeca have two functions: the blind end area contains regenerative cellular nests, whereas the ones constituting the connecting part with the midgut are typical digestive glands. Therefore the gastric caeca can be considered as digestive glands.

The midgut epithelium is mainly made of columnar cells. In spite of their common morphological organization, they have different functions in the anterior and posterior midgut regions indicating some kind of functional bipolarity. We think that the anterior region plays a role in secretion and absorption as the digestive hydrolases are mainly detected in the anterior epithelium (Table 1). Moreover, the thickness of the glycocalyx and the detection of oligosaccharidases at the cell top suggest a process of "contact-digestion", at least for oligomeres. Thus absorption could occur with "terminal digestion" as is found in vertebrates (Ugolev 1965, Barnard 1973) and in some insects, such as Diptera (Terra et al. 1979).

The posterior region of the midgut can be considered as the site of intracellular storage processes, excretion and hydromineral regulation. Hydrolase activity is generally low in this region.

3. Hindgut

The hindgut consists of two regions: the ileum and the rectum . In the latter, six rectal papillae can be seen.

The cells of ileum and rectal pads display an ultrastructural organization typical of absorptive epithelia. Such features include: apical and basal infolds of plasma membranes closely associated with numerous mitochondria, fine particulate coating on the cytoplasmic surface of apical plasma membranes, scalariform junctions in the rectal cells and fine tracheoles in the intercellular spaces of the ileum cells. All these ultrastructural features are generally related to hydromineral regulation (Martoja and Ballan-Dufrancais 1984). The rectal pads are surrounded by a rectal epithelium displaying ultrastructural organization common to other "mechanical epithelia", like that in the foregut.

Digestive hydrolases were detected in the proctodeal lumen but never close to the epithelium. The enzymes actually originate from the midgut,

Table 1

Activities of chitinase, cellulase and proteases in seven carabid species, detected by histochemical methods. Numbers are histoenzymological units (Jaspar-Versali 1985)

Species	Chitinase				Cellulase				Proteases			
	fore-gut	anterior midgut	posterior midgut	hind-gut	fore-gut	anterior midgut	posterior midgut	hind-gut	fore-gut	anterior midgut	posterior midgut	hind-gut
C. splendens	8.7	7.4	4.9	1.9	1.6	2.3	2.3	1.9	7.4	6.2	5.6	2.2
C. nemoralis	6.8	6.3	4.0	2.3	2.3	4.3	4.3	3.5	7.4	5.6	5.6	1.0
C. arvensis	6.1	6.3	4.1	1.0	1.6	4.3	4.3	1.0	8.5	7.7	5.6	1.5
C. problematicus	9.0	6.1	4.4	2.3	1.6	2.3	2.3	1.6	7.7	7.4	6.2	1.0
C. cancellatus	7.4	6.9	4.9	2.1	1.6	2.0	1.6	1.0	8.9	8.5	5.6	1.8
A. paralellepipedus	7.1	7.1	5.1	2.3	6.0	7.0	7.0	1.6	3.8	3.3	2.2	1.0
P. melanarius	6.3	7.1	4.9	2.8	7.0	8.0	8.0	1.6	5.1	4.4	1.5	1.0

their activities are low in the hindgut (Table 1); this is probably due to some kind of alteration during the intestinal transit.

CONCLUSIONS

Taken on the whole, the present ultrastructural observations show that the digestive tract of all the carabid species we have examined exhibits a remarkably similar organization.Except for some secondary structures (such as teeth, setae, ...), it displays few specialized features and therefore can be regarded as a primitive system similar to that described in other primitive adult insects as well as in many larvae (Wigglesworth 1965).

From a biochemical point of view, carabid beetles are characterized by a large spectrum of digestive enzymes, including chitinase, cellulase, amylase, laminarinase, proteases and various oligosaccharidases. Taking into consideration that these enzymes are constituents of the ancestral equipment of digestive hydrolases of the animal cell (Yokoe and Yasumasu 1964, Jeuniaux 1963, Piavaux 1977, Jaspar-Versali and Jeuniaux in press), the presence of a diversified digestive enzymatic equipment in carabids can be considered as a primitive character.

The primitive organization of the alimentary canal and broad enzyme spectrum, the morphological and functional features of the digestive system and the opportunistic feeding behaviour of carabids are as many factors that have probably contributed to widen their distribution in terrestrial habitats.

ACKNOWLEDGMENTS

This work was supported by the "Fonds de la Recherche Fondamentale Collective" of the Belgian Fund for Scientific Research (number 2.4506.83).

LITERATURE

Arnould, Ch. and Bouchez-Decloux, N. (1978): Méthodes histoenzymologiques pour la détection de cellulase, de chitinase et de laminarinase. Application au bouclier gastrique du mollusque bivalve Scrobicularia plana. Histochemistry, 56, 45-54.

Balfour-Browne, F. (1944). The proventriculus of the coleoptera (Adephaga) and other insects. A study in evolution. Journal of the Royal Microscopical Society, 64, 68–117.

Barnard, E.A. (1973): Comparative biochemistry of digestive enzymes. Comparative Animal Physiology (Ed. by C.L. Prosser), pp. 139–146. Saunders, Philadelphia.

Caveney, S. (1969). Muscle attachment related to cuticle architecture in Apterygota. Journal of Cell Science, 4, 541–559.

Cheeseman, M.T. and Pritchard, G. (1984a): Spatial organization of digestive processes in an adult carabid beetle, Scaphinotus marginatus (Coleoptera: Carabidae). Canadian Journal of Zoology, 62, 1200–1203.

Cheeseman, M.T. and Pritchard, G. (1984b): Proventricular trituration in adult carabid beetles (Coleoptera: Carabidae). Journal of Insect Physiology, 30, 203–209.

Compere, P. and Goffinet, G. (1987): Aspects ultrastructuraux et fonctionnels de diverses régions cuticulaires non minéralisées d'un Crustacé Décapode, Carcinus maenas. Annales de la Société royale Zoologique de Belgique (in press).

Hayashi, M. (1965): Histochemical demonstration of N-acetyl-β-glucosaminide as substrate. Journal of Histochemistry and Cytochemistry, 13, 355–360.

House, H.L. (1974): Digestion. The Physiology of Insecta (Ed. by Rockstein), vol. V, pp. 63–117. Academic Press, New York and London.

Jaspar-Versali, M.F. (1985): Le tube digestif et les processus de la digestion chez les Coléoptères Carabides, Pterostichides et quelques autres familles: étude morphologique et histoenzymologique comparée. Unpublished Ph. D. Thesis (in French), University of Liege.

Jaspar-Versali, M.F., Bouchez-Decloux, N. and Bussers, J.C. (1982): Localisation histoenzymologique des chitinases, cellulases et protéases chez un Coléoptère carabique: Abax ater (villers, 1789). Annales de la Société royale Zoologique de Belgique, 112(2), 147–156.

Jaspar-Versali, M.F. and Jeuniaux, Ch. (1987): L'équipement eznymatique digestif des Carabes envisagé sous l'angle de leur niche écologique. Proceedings du Colloque "Fonctionnement du sol et Systemes biologiques interactifs (Paimpont, 1986) (in press).

Jaspar-Versali, M.F., Poulicek, M. and Goffinet, G. (1985): Le stomodeum des Carabes: essai d'interprétation fonctionnelle. Annales de la Société royale Zoologique de Belgique, 115(1), 87–88.

Jaspar-Versali, M.F., Poulicek, M., Toussaint, C. and Goffinet, G. (1985): Microstructures du tractus digestif et transit intestinal chez quelques Caraboidea. Annales de la Société royale Zoologique de Belgique, 115(1), 104–105.

Jeannel, R. (1941): Faune de France. Coléoptères Carabiques. (Ed. by Lechevalier et Fils), Tomes 1 and 2.

Jeuniaux, Ch. (1963): Chitine et chitinolyse. Un chapitre de la biologie moléculaire (Ed. by Masson).

Jeuniaux, Ch. (1971): Hemolymph Arthropoda. Chemical Zoology (Ed. by Florkin and Scheer), vol. 5, pp. 64–118. Academic Press.

Lojda, Z. (1972): An improved histochemical method for the demonstration of disaccharidases with natural substrates. Histochemie, 30, 277–280.

Martoja, R. and Ballan-Dufrançais, C. (1984): The ultrastructure of the digestive and excretory organs. Insect Ultrastructure (Ed. by King and Akai), vol. 2, pp. 199–268. Plenum Press, New York and London.

Michel, C. and Chretien, M. (1975): Modification of Cunningham's method for the detection of proteases in tissue sections. Annales d'Histochimie, 20, 39–46.

Piavaux, A. (1977): Distribution and localization of the digestive laminarinases in Animals. Biochemical Systematics and Ecology, 5, 231–239.

Rutenburg, A.M., Goldberg, J.A., Rutenburg, S.H. and Lang, T.R. (1960): The histochemical demonstration of α-D-glucosidase in mammalian tissues. Journal of Histochemistry and Cytochemistry, 8, 268–272.

Schlottke, E. (1937): Untersuchungen über die Verdauungsfermente von Insekten. Die Verteilung der Fermente im Darmkanal von fleischfressenden Carabiden und die Änderungen ihrer Konzentration während der Verdauung. Zeitschrift für Vergleichende Physiologie, 24, 210–247.

Skuhravy, V. (1959): Die Nahrung der Feldcarabiden. Acta Societatis Entomologicae Cechosloveniae, 56, 1–18.

Spence, J.R. and Sutcliffe, J.F. (1982): Structure and function of feeding in larvae of Nebria (Coleoptera: Carabidae). Canadian Journal of Zoology, 60, 2382–2394.

Terra, W.R., Ferreira, C. and Debianchi, A.G. (1979): Distribution of digestive enzymes among the endoperitrophic and ectoperitrophic spaces and midgut cells of Rhynchosciara americana and its physiological significance. Journal of Insect Physiology, 25, 487–494.

Thiele, H.U. (1977): Carabid beetles in their environments. A study on habitat selection by adaptations in physiology and behaviour. Springer Verlag, Berlin.

Ugolev, A.M. (1965): Membrane (contact) digestion. Physiological Reviews, 45, 555–595.

Wigglesworth, V.B. (1965): The principles of Insect Physiology, 6th ed., pp. 427–495. Dutton, New York.

Wigglesworth, V.B. (1972): The principles of Insect Physiology, 7th ed., Chapman and Hall, London.

Yokoe, Y. and Yasumasu, I. (1964): The distribution of cellulase in invertebrates. Comparative Biochemistry and Physiology, 13, 323–338.

Zhavoronkova, T.N. (1969): Certain structural peculiarities of the Carabidae (Coleoptera) in relation to their feeding habits. Entomological Reviews, 48, 426–471.