Histological organization of the intestine in the crayfish *Procambarus clarkii*

Terence H. To, Tracy L. Brenner, Michael J. Cavey and Jerrel L. Wilkens

Department of Biological Sciences, Division of Zoology, University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4

Abstract

To, T. H., Brenner, T. L., Cavey, M. J. and Wilkens, J. L. 2004. Histological organization of the intestine in the crayfish *Procambarus clarkii*. — *Acta Zoologica* (Stockholm) **85**: 119–130

Six longitudinal ridges span the length of the intestine in the crayfish *Procambarus clarkii*. A simple columnar epithelium with tetralaminar cuticle lines the lumen. Folds of the epithelium overlie a dense irregular connective tissue packed with mixed acinar (alveolar) glands. Mucous secretions are probably involved with formation and lubrication of faecal strings; neither the nature nor the role of the serous secretions is immediately apparent. Aggregations of cells with large cytoplasmic vacuoles, called bladder cells, appear in the subepithelial connective tissue near the tops of the intestinal ridges. The bladder cells are suitably positioned to bolster the integrity of the ridges. Striated muscle of the intestine occurs in inner longitudinal and outer circular layers. The inner longitudinal layer consists of six strips, with one strip associated with the base of each intestinal ridge. The outer circular layer is essentially complete, but there are periodic apertures in this layer on the left and right sides of the intestine, providing nerves and haemolymph vessels with access to the interior of the gut. Based on histological features, and consistent with reports on other crayfish, we conclude that the intestine of *P. clarkii* has a proctodeal (ectodermal) origin.

Dr Michael J. Cavey, Department of Biological Sciences, Division of Zoology, University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4. E-mail: mcavey@ucalgary.ca

Introduction

The alimentary viscus of a decapod crustacean arises from an ectodermal stomodeum, an endodermal mesenteron, and an ectodermal proctodeum (Yonge 1924; Vonk 1960; Shiino 1968; Burrage 1978; Johnson 1980). The foregut (mouth, oesophagus, cardiac stomach and pyloric stomach) derives from the stomodeum; stomodeal derivatives are lined by a chitinous cuticle. The midgut [hepatopancreas, midgut caeca and (in some decapods) intestine] arises from the mesenteron; derivatives of the mesenteron lack a cuticle on the adluminal epithelium. The hindgut [rectum and (in some decapods) intestine] derives from the proctodeum; proctodeal derivatives, like those of the stomodeum, are lined by a chitinous cuticle (Yonge 1924; Mykles 1979; Factor 1981b, 1995).

There is a small body of structural data on mesenteron-derived and proctodeum-derived intestines of decapods. In the lobster *Homarus americanus*, a caecum in the sixth abdominal segment marks the posterior end of the midgut (Factor 1995). Freshwater crayfish, such as *Procambarus*

clarkii, lack such a midgut caecum (Smith 1978). Distinctions between mesenteron-derived and proctodeum-derived intestines are based essentially on the presence or absence of caeca and on the presence or absence of a cuticle next to the lumen (Huxley 1880; Lochhead 1950; Devi *et al*. 1989; Trinadha Babu *et al*. 1989).

The disposition of the intestinal muscle also appears to be a well-conserved feature among decapod crustaceans. In a mesenteron-derived intestine, muscle cells appear in inner circular and outer longitudinal layers. The reverse configuration is found in a proctodeum-derived intestine, where muscle cells appear in inner longitudinal and outer circular layers (Dall 1967; Komuro and Yamamoto 1968; Winlow and Laverack 1972a; Factor 1979, 1995; Johnson 1980; Devi *et al*. 1989; Trinadha Babu *et al*. 1989). In a proctodeum-derived intestine, muscle cells from the inner layer project into longitudinal folds of the intestinal wall (Frenzel 1885; Yonge 1924; Pillai 1960; Barker and Gibson 1977).

The goals of our study were to conduct a comprehensive examination of the histology of the intestine of *P. clarkii* that

would complement recent physiological findings; to compare intestinal features of this freshwater crayfish with those of other decapod crustaceans; and to compile a list of attributes warranting further examination by transmission electron microscopy. As part of the histological survey, a fluorescent phallotoxin (rhodamine phalloidin) was used to label actin-containing filaments in cells of the intestine. Aspects of this study have been reported in an abstract (To *et al*. 1999).

Materials and Methods

Animals and dissection

Large crayfish (*Procambarus clarkii* Girard 1852) of both sexes were obtained from The Atchafalaya Biological Supply Company in Raceland, LA, USA, maintained in aquaria at 22 °C, and fed a diet of dog food. Animals were pithed prior to dissection. The intestine was harvested under saline (Van Harreveld 1936) by making two anteroventral incisions from the end of the cephalothorax to the telson. The carapace was lifted off, and the ventral segmental muscles were trimmed away to expose the underlying intestine. The ventral nerve cord and the dorsal arteries were excised carefully from the intestine. The intestine was then flushed with saline and removed by making incisions at the cephalothorax–abdomen junction and at the sixth abdominal segment.

Histology

A sector of intestine near the stomach was cut into short cylindrical segments with razor blades, and the segments were immersed in fixative [2.5% glutaraldehyde in 0.2 M Millonig's phosphate buffer (pH 7.4), containing 0.14 M sodium chloride; Cloney and Florey 1968] for 2 h. The specimens were then dehydrated with a graded series of ethanol and infiltrated and embedded with glycol methacrylate, using a Reichert–Jung Historesin kit (Yeung and Law 1987). Sections (3 μm in thickness) were cut with glass (Ralph) knives on a Microm HM 330 rotary microtome and stained with a solution of 0.5% azure II and 0.5% methylene blue in 0.25% sodium borate at room temperature (Cavey *et al*. 1992). Cover glasses were mounted with Fisher Permount.

The sections were viewed and photographed with a Nikon Optiphot compound microscope equipped with planachromatic objective lenses and an HFX-II photomicrographic attachment. The microscope was calibrated with a stage micrometer (100 lines/mm). Kodak Panatomic-X film was over-exposed and under-developed to reduce the size of silver grains in the finished negatives.

Immunohistochemistry

Intestinal segments were cut open lengthwise and transferred to cover glasses. The spreads were ringed with petroleum jelly, washed with saline (Cole 1941), fixed with 3.7% formaldehyde in saline (10 min), washed in saline (2 × 1 min), permeabilized with 0.1% Triton X-100 in saline (5 min), washed in saline (2 × 1 min), and incubated in 1% bovine serum albumin in saline (30 min). Double-strength rhodamine phalloidin stock (R-415; Molecular Probes, Eugene, OR, USA) was added to the reservoir for 20 min. After staining, the spreads were washed in saline (2 × 1 minute), and cover glasses were mounted on microscope slides with 1 : 1 glycerol : saline. As a control, the rhodamine phalloidin stock was omitted from the protocol.

The whole mounts of intestine were viewed and photographed with a Nikon Eclipse TE300 inverted fluorescence microscope. The microscope was calibrated with a stage micrometer (100 lines/mm). Kodak T-Max 100 film was exposed and processed according to the manufacturer's recommendations.
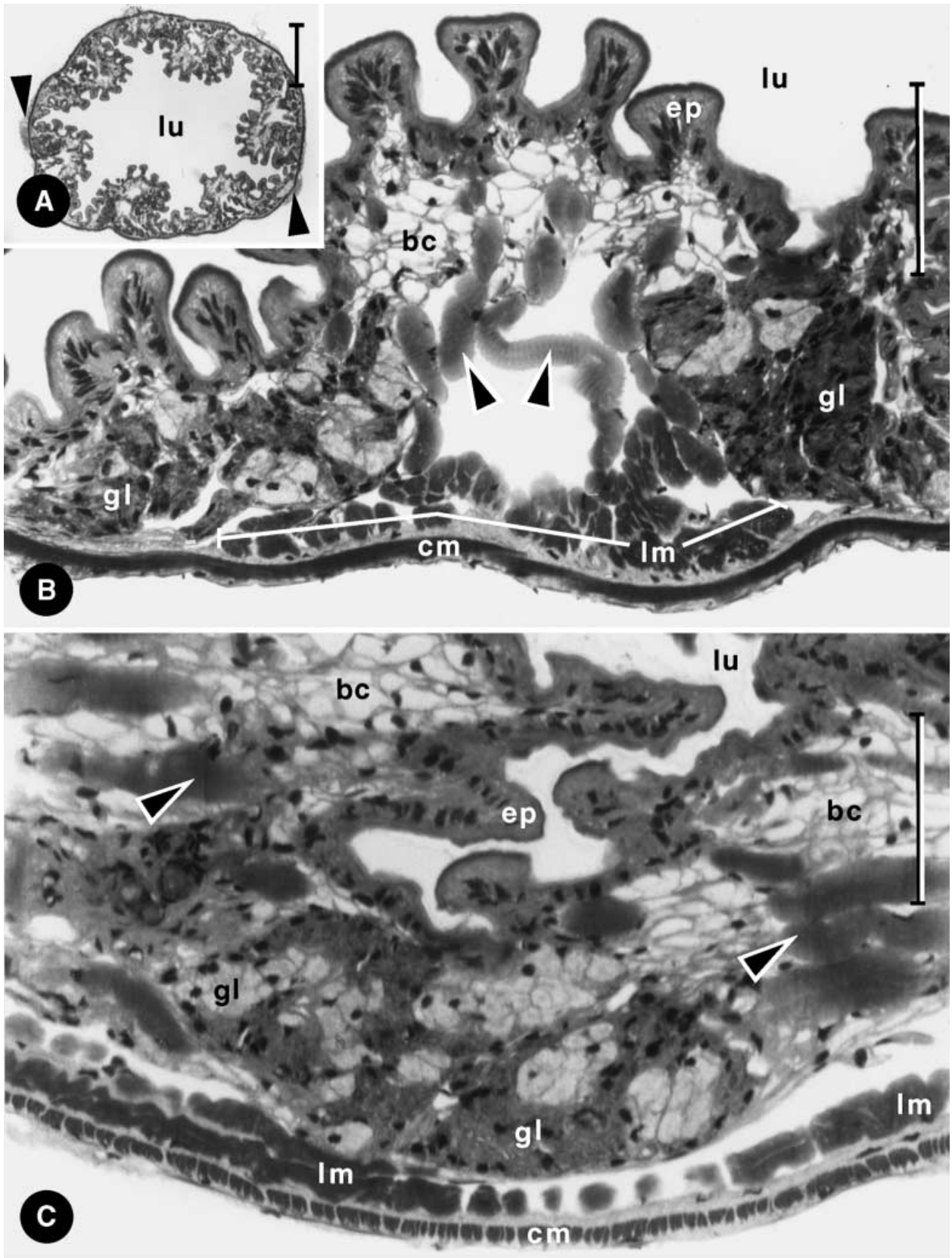
Results

The intestine of *P. clarkii* has a circular or oblong outline in transverse sections (Fig. 1A). Six longitudinal ridges project inward from the wall of the organ, giving the lumen a stellate appearance. Each ridge consists of an adluminal epithelium and a subepithelial connective tissue with glands. The remainder of the intestinal wall is configured of inner longitudinal and outer circular layers of striated muscle, which are embedded in a connective tissue that also forms the external boundary of the viscus (Fig. 1B,C).
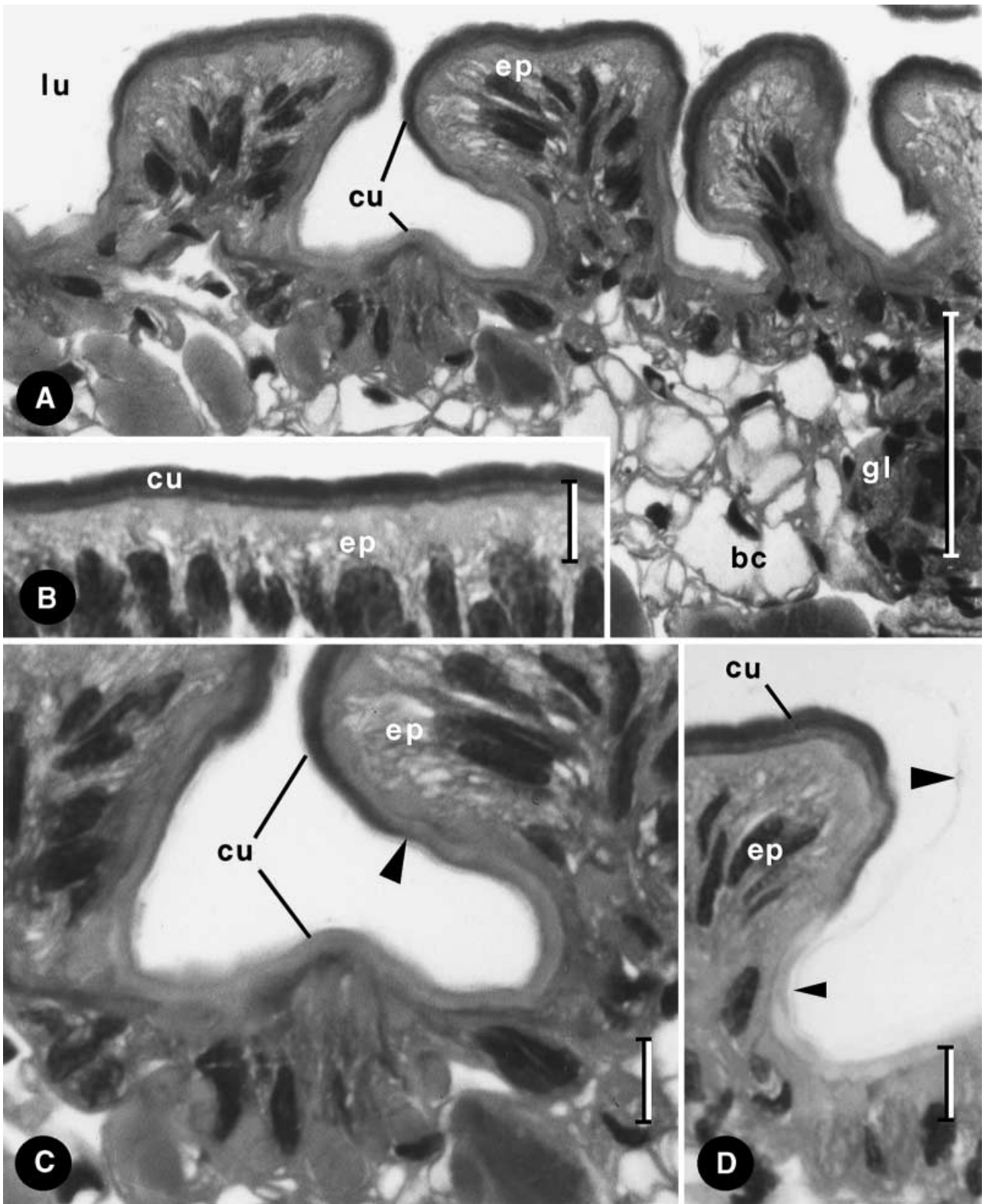
Adluminal epithelium and subepithelial connective tissue

Tissues flanking the intestinal lumen are a simple columnar epithelium and a dense irregular connective tissue (Fig. 1B,C). Epithelial folds are prominent, both on the intestinal ridges and in the depressions between ridges (Fig. 2A). There is no conspicuous basement membrane at the epithelium–connective tissue interface.

Fig. 1—Intestine of *Procambarus clarkii*. —**A**. Transverse section of the viscus. Six ridges impinge on the lumen. Masses of nerves and haemolymph vessels (arrowheads) flank the left and right sides of the organ. —**B**. Transverse section of an intestinal ridge. Acinar glands situated in the subepithelial connective tissue in the depressed sectors between ridges. A longitudinal strip of striated muscle cells occurs in the base of each ridge, and fascicles of cells (arrowheads) from the inner aspect of this strip span obliquely toward the overlying epithelium. —**C**. Longitudinal section of the viscus. The secretory units of acinar glands are prevalent in the subepithelial connective tissue, especially in depressed sectors of the wall; bladder cells aggregate in elevated sectors of the wall. Fascicles of longitudinal muscle cells (arrowheads) pass between the bladder cells to approach the adluminal epithelium. Bars: —**A** 250 μm; —**B**, **C** 100 μm; *bc*, bladder cells; *cm*, circular layer of striated muscle; *ep*, adluminal epithelium; *gl*, acinar glands; *lm*, longitudinal layer of striated muscle; *lu*, intestinal lumen.

Cells at the tops and on the sides of the epithelial folds are columnar in shape, and their elongate nuclei are restricted to the basal cytoplasm (Fig. 2C). Epithelial cells at the tops of the folds tend to be taller than those on the sides. Epithelial cells in both locations have a fibrous cytoplasm, and their nuclei are mottled by condensed chromatin.

A tetralaminar cuticle appears on the adluminal epithelium (Fig. 2B). The superficial lamina of this cuticle is a thin, pale epicuticle. A thick, dense exocuticle lies beneath the epicuticle, and the exocuticle is adjoined by a thick, pale endocuticle. The deep lamina of the cuticle is a thin, moderately dense, membranous layer in contact with the apical surface of the epithelium (terminology after Waddy *et al.* 1995). The exocuticle is a robust lamina at the tops and along the apicolateral surfaces of the epithelial folds. On the sides of the folds, the exocuticle becomes quite thin and, thus, rather inconspicuous along the basolateral surfaces of the folds and in the depressions between folds. Thickness of the exocuticle correlates with obvious differences in resin penetration during infiltration (Fig. 2D). In areas where the exocuticle is thickest, the glycol methacrylate fails to penetrate the cuticle. Enhanced penetration by the resin is evident in those sectors of cuticle where the exocuticle is thinnest.

Mixed acinar (alveolar) glands are situated in the subepithelial connective tissue on the sides of the intestinal ridges and in the depressions between ridges (Fig. 3B). Some secretory units appear to consist of mucous cells alone (Fig. 3C), and others seem to contain just serous cells (Fig. 3D). In both mucous cells and serous cells, the nuclei are positioned toward the periphery of a secretory unit. The nuclei of mucous cells are slightly more flattened than those of serous cells. Mucigen droplets and zymogen granules are well preserved by the histological technique. Smaller ducts of the subepithelial glands emerge from the secretory units (Fig. 3B–D). Openings of the ducts into the intestinal lumen were not observed. It is possible that the ducts project forward to open into the anteromost sector of intestine, as has been described for the crabs *Scylla serrata* (Barker and Gibson 1978) and *Menippe rumphii* (Erri Babu *et al.* 1982).

A peculiar cell type appears in the subepithelial connective tissue near the top of each intestinal ridge. It is a massive, vacuolate cell with a peripheral ovoid nucleus that we are calling a 'bladder cell' (Figs 1B and 3A). Owing to its restricted position, a bladder cell may be a supportive element that deters the collapse of a ridge as faeces are formed in and

transported along the lumen. Long strings of faeces, consisting of small particles embedded in mucus, appear to be formed and expelled continually by this crayfish. The faecal strings have a distinctive pattern of longitudinal grooves, probably sculpted by the inward-projecting ridges of the intestinal wall.

Musculature and perimuscular connective tissue

The intestine of *P. clarkii* has inner longitudinal and outer circular layers of striated muscle (Fig. 1B,C). The inner layer consists of six longitudinal strips, with one strip associated with the base of each intestinal ridge. Longitudinal muscle cells are larger than circular muscle cells (Fig. 4A). The longitudinal cells are multinucleate. We could not ascertain, on the basis of histological sections alone, if the circular cells are uninucleate or multinucleate. The sarcomeres of a circular muscle cell are wider and more variable in width than those of a longitudinal muscle cell. Dispositions of the striated muscle cells have been verified by immunohistochemical staining for actin in the thin myofilaments of the sarcomeres (Fig. 5A, B). No other cells of the intestine show appreciable amounts of filamentous actin.

The longitudinal muscle cells are enveloped by a dense irregular connective tissue. Cells frequently emerge from the internal aspect of a longitudinal muscle strip, span obliquely into the supra-adjacent intestinal ridge (Fig. 4B), and infiltrate between the bladder cells (Fig. 3A) before inserting near the boundary of adluminal epithelium and subepithelial connective tissue. In histological sections of longitudinal muscle cells spanning to their sites of insertion, one can identify endomysium-like connective-tissue sheaths around individual cells and perimysium-like sheaths surrounding fascicles of cells. In transverse sections of the intestine, oblique and longitudinal views of the longitudinal muscle cells are often apparent in the intestinal ridges (Fig. 2A). We find no evidence that connective-tissue fibres around the muscle cells penetrate the adluminal epithelium to insert on the cuticle (Frenzel 1885; Yonge 1924) or that muscle cells form direct attachments to the adluminal epithelium (Vitzou 1882).

The circular muscle band approximates the external boundary of the viscus (Fig. 4D). Thin layers of connective tissue separate adjacent cell bundles, and slightly thicker connective-tissue masses segregate the circular muscle layer from each longitudinal muscle strip, as well as from the exterior edge of

---

Fig. 2—Adluminal epithelium in the intestine of *Procambarus clarkii*. —**A**. Folds of the simple columnar epithelium and the dense irregular connective tissue. A cuticle appears on the apical surface of the adluminal epithelium. Bladder cells and acinar glands occupy the subepithelial connective tissue. —**B**. Cuticle on the top of an epithelial fold. The cuticle consists of a superficial epicuticle exposed to the lumen, an exocuticle, an endocuticle, and a deep membranous layer in contact with the epithelium. —**C**. Cuticle over an epithelial fold and in the depression between folds. The exocuticle

thins abruptly (arrowhead) on the lateral surface of a fold. —**D**. Failure of glycol methacrylate to infiltrate the cuticle where the exocuticle is thickest. Observe the retraction of the resin at the top and the apicolateral surface (large arrowhead) of the fold. Retraction is less pronounced at the basolateral surface (small arrowhead) of the fold and still less pronounced or absent entirely over epithelial sectors in the depressions between folds. Bars: —**A** 50 μm; —**B**–**D** 10 μm; *bc*, bladder cells; *cu*, cuticle; *ep*, adluminal epithelium; *gl*, acinar glands; *lu*, intestinal lumen.
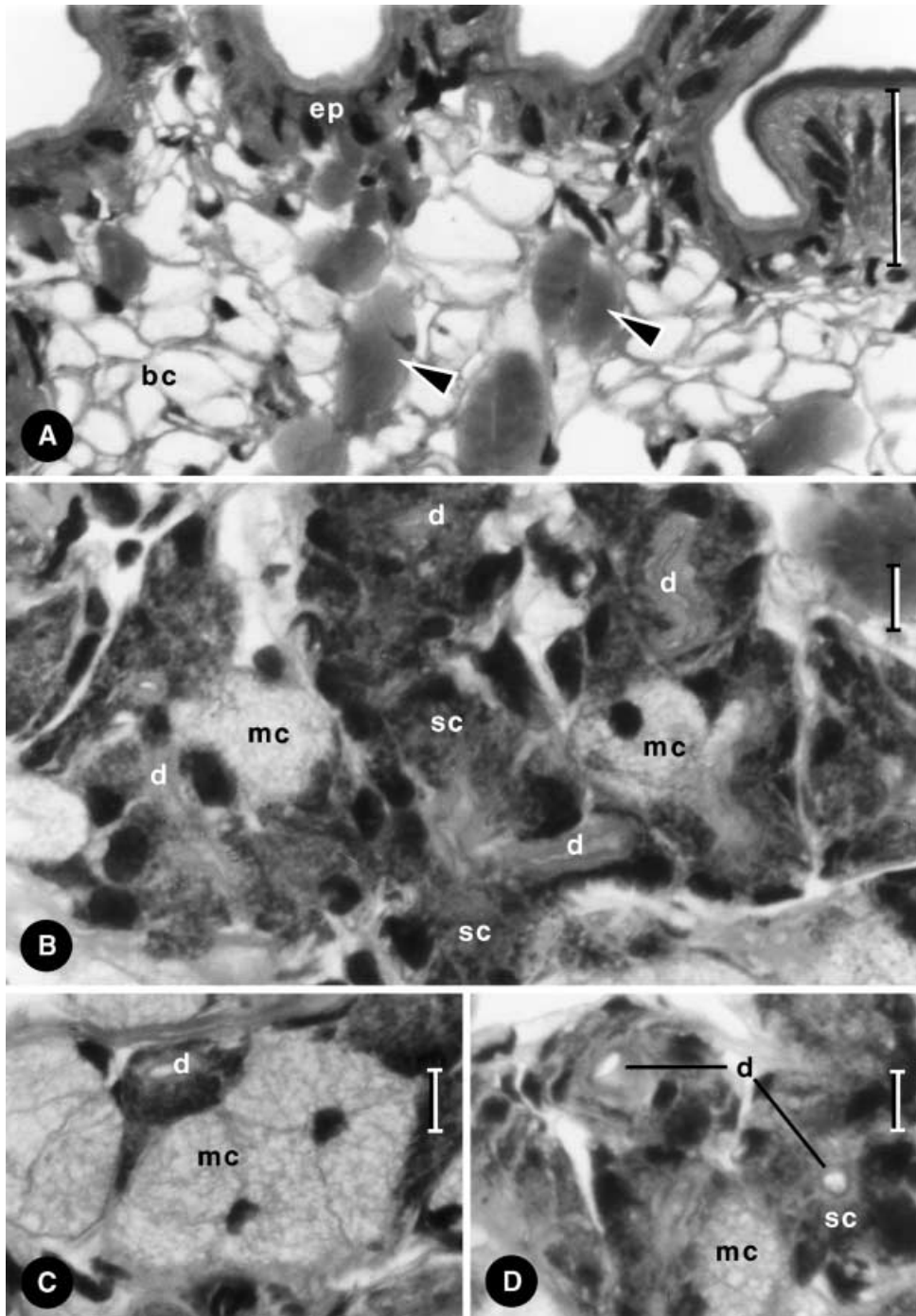
Fig. 3—Subepithelial connective tissue in the intestine of *Procambarus clarkii*. —**A**. Aggregated bladder cells beneath the adluminal epithelium in an intestinal ridge. A pale precipitate appears in the cytoplasmic vacuoles. Fascicles of striated muscle cells (arrowheads), originating from a longitudinal strip, insert on the subepithelial connective tissue between the bladder cells and the adluminal epithelium. —**B**. Mixed acinar glands in the subepithelial connective tissue. Mucous cells and serous cells populate the acini, and their nuclei tend to be situated near the peripheries of the secretory units. Several ducts are evident in the field. —**C**. Mucous cells of the subepithelial acini and small secretory duct. Mucigen droplets are readily resolved in the cytoplasm of a mucous cell. —**D**. Small secretory ducts emerging from serous acini in the subepithelial connective tissue. Zymogen granules are readily resolved in the cytoplasm of a serous cell. Bars: —**A** 50 μm; —**B**–**D** 10 μm; *bc*, bladder cells; *d*, duct; *ep*, adluminal epithelium; *mc*, mucous cells; *sc*, serous cells.
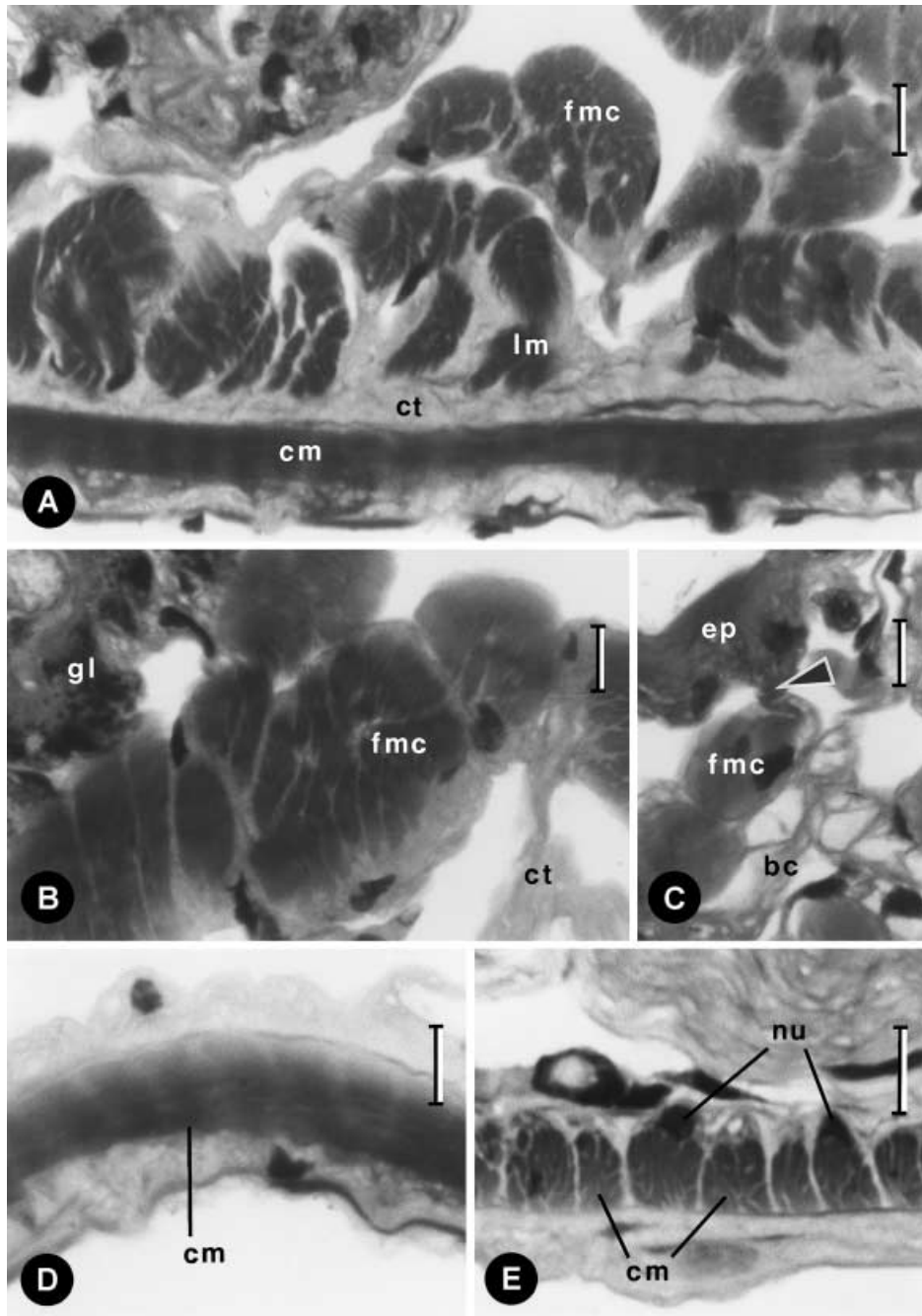
Fig. 4—Muscle layers in the intestine of *Procambarus clarkii*. —**A**. Transverse section of the inner and outer layers of striated muscle. Connective tissue binds the muscle cells together. Fascicles of muscle cells, also invested by connective tissue, leave the longitudinal strip and project obliquely toward the adluminal epithelium. —**B**. Fascicle of muscle cells near the internal edge of a longitudinal strip. Note the connective-tissue sheaths around and between cells of a fascicle. —**C**. Continuity (arrowhead) of connective tissues underlying the adluminal epithelium and surrounding a fascicle of longitudinal muscle cells. Terminal regions of a fascicle penetrate between bladder cells to insert on the subepithelial connective tissue adjacent to the basement membrane. —**D**. Transverse section of the outer layer of striated muscle. Sarcomeres of the circularly orientated cells are wider than those of the longitudinally orientated cells and more irregular in width. —**E**. Longitudinal section of the outer layer of striated muscle. Note the union of several cells by connective tissue to form a bundle. Nuclei of circular muscle cells tend to localize at the tapered (external) edges of the bundles. Bars: —**A**–**E** 10 μm; *bc*, bladder cells; *cm*, circular layer of striated muscle; *ct*, connective tissue; *ep*, adluminal epithelium; *fmc*, fascicle of striated muscle cells; *gl*, acinar glands; *lm*, longitudinal layer of striated muscle; *nu*, nucleus.
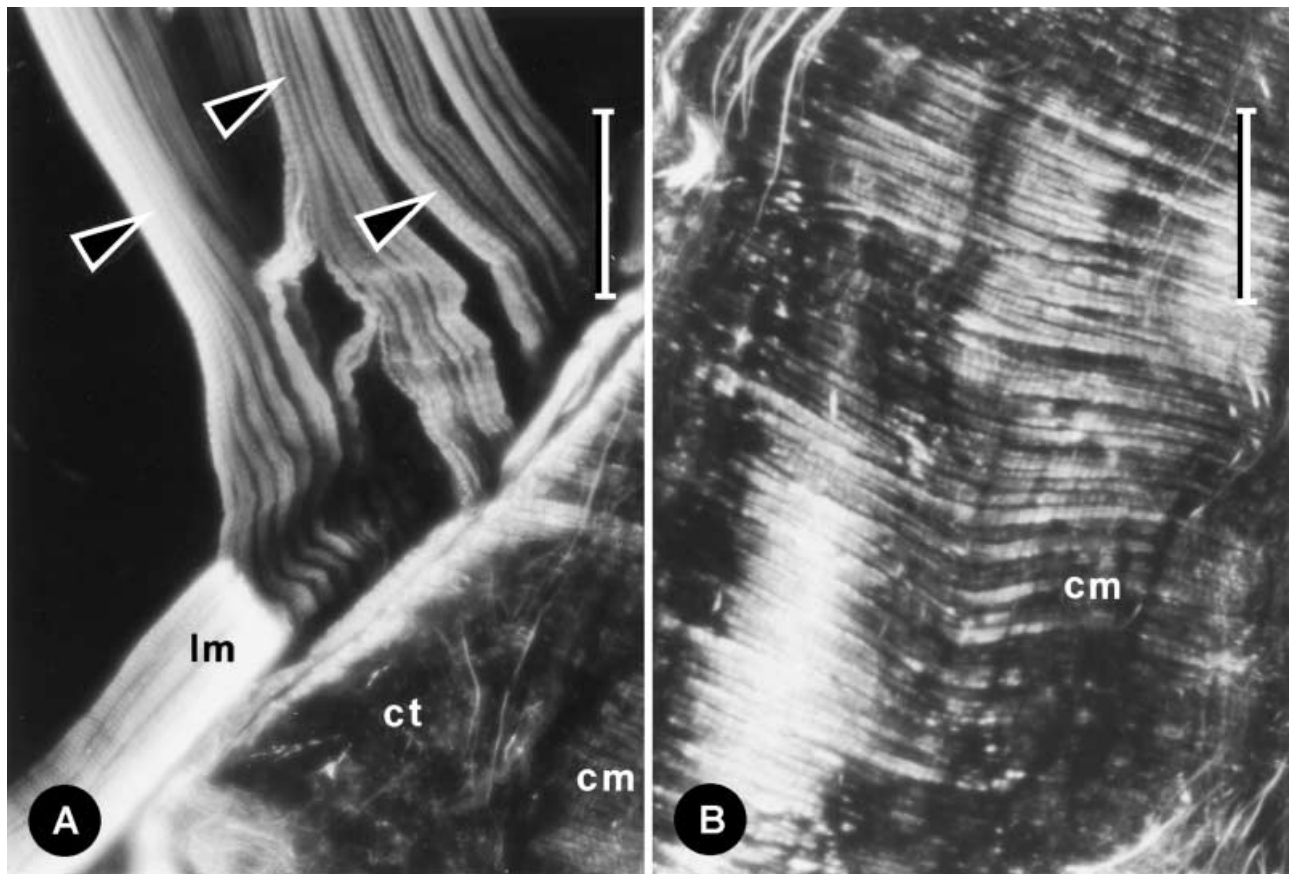
Fig. 5—Immunohistochemical staining for F-actin in a whole mount of the intestine of *Procambarus clarkii*. —**A**. Fascicles of striated muscle cells (arrowheads) from an inner longitudinal strip span obliquely toward the apex of an intestinal ridge (cf. Fig. 1B). A modest layer of connective tissue embeds the outer circular layer of striated muscle and segregates it from the six longitudinal strips. —**B**. Striated muscle cells in the outer circular layer. Observe the close packing of the bundles. Bars: —**A**, **B** 100 μm; *cm*, circular layer of striated muscle; *ct*, connective tissue; *lm*, longitudinal layer of striated muscle.

the intestine. Densely stained, elongate nuclei are visible at the periphery of each bundle of muscle cells. In longitudinal sections, the outlines of the circular muscle bundles appear triangular or wedge-shaped (Fig. 4E). Flattened surfaces of the bundles face inward, and the tapered surfaces point outward (Fig. 1C).

The posterior intestinal nerves, originating from the sixth abdominal ganglion (Florey 1961; Winlow and Laverack 1972b,c), and haemolymph vessels are evident along the left and right sides of the viscus (Fig. 6A). At intervals along the length of the intestine, apertures appear in the outer circular layer of muscle (Fig. 6B), giving nerves and vessels access to the connective tissue between the circular and longitudinal layers of muscle.

Discussion

Six longitudinal ridges appear along the hindguts of the crab *Cancer magister*, the lobsters *Homarus americanus* and *Nephrops norvegicus*, and the shrimp *Caridina laevis* (Yonge 1924;

Pillai 1960; Johnson 1980; Factor 1995). The intestine of *P. clarkii* similarly shows six intestinal ridges projecting deep into the lumen (Komuro and Yamamoto 1968; present study).

Intestinal function

In addition to structural differences, there are also physiological differences between the midguts and the hindguts of decapod crustaceans. The adluminal epithelium of the midgut, for example, contains absorptive cells, and it may incorporate ionoregulatory cells as well (Komuro and Yamamoto 1968; Talbot *et al.* 1972; Mykles 1977, 1979; Smith 1978). The hindgut, including the intestine of decapods such as the crayfish *Astacus fluviatilis* and some brachyurans, is specialized for secretion and usually exhibits well-developed subepithelial (tegumental) glands (Smith 1978). The hindgut is believed to function in the packaging, lubrication and transportation of faeces (Erri Babu *et al.* 1982; Devi *et al.* 1989). The intestine of *P. clarkii* is spontaneously active and contracts by peristalsis, a moving wave of contraction generated
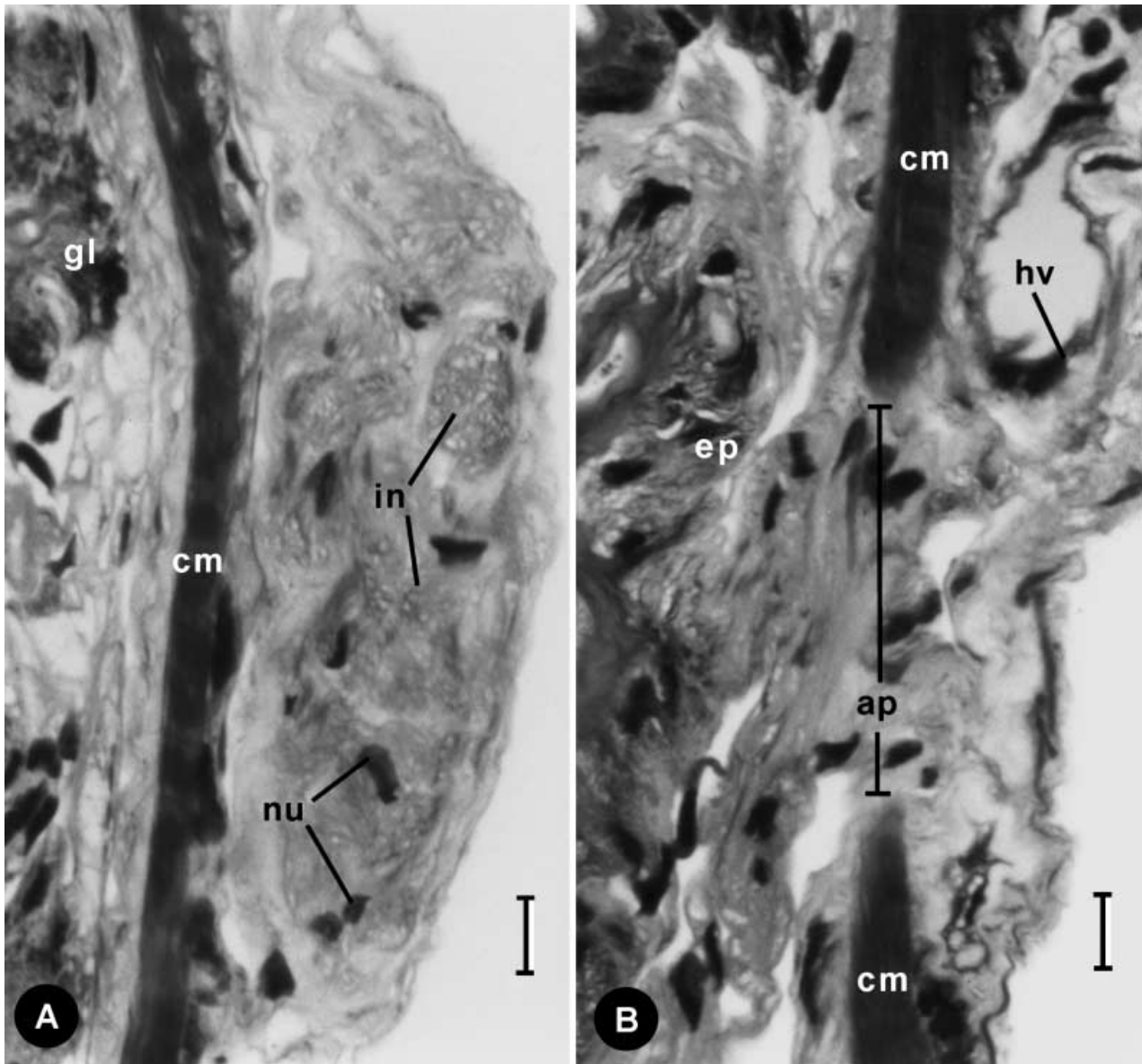
Fig. 6—Nervous and vascular elements serving the intestine of *Procambarus clarkii* (cf. Fig. 1A). —**A**. Lateral mass of intestinal nerves and haemolymph vessels situated close to the outer circular layer of striated muscle. Nuclei associated with the intestinal nerves belong to glial cells. Acinar glands appear in the subepithelial connective tissue. —**B**. Aperture in the outer circular layer of striated muscle admitting nerves and haemolymph vessels to the interior of the viscus. The adluminal epithelium, occurring in a depression between intestinal ridges, closely approaches the outer circular layer of muscle. Bars: —**A**, **B** 10 μm; *ap*, aperture; *cm*, circular layer of striated muscle; *ep*, adluminal epithelium; *gl*, acinar glands; *hv*, haemolymph vessel; *in*, intestinal nerve; *nu*, nucleus.

by the longitudinal muscle strips that is easily visualized both *in situ* and *in vivo* (Florey 1961; J. Mercier, personal communication). Each intestinal ridge evidently contains a pacemaker, because the ridges can be excised from the intestine, and they continue to show spontaneous peristaltic activity (cf. Ebara 1969; Brenner 1999). Should an inner strip of muscle be cut when excising a ridge, organized contractile waves in that ridge are either interrupted or eliminated. Such observations suggest that each intestinal ridge is a discrete functional unit. The abolition of contractility when the muscle bundle inside a ridge is cut implies the presence of electrical coupling, perhaps via communicating (gap) junctions. The presence of communicating junctions in the embryonic gut (between epithelial cells and between muscle cells) of *Homarus americanus* (Burrage 1978) increases the likelihood that they occur in the intestine of *P. clarkii*.

Adluminal epithelium and subepithelial connective tissue

There does not appear to be a prominent basement membrane at the interface between adluminal epithelium and subepithelial connective tissue in the intestine of *P. clarkii*. In the lobsters *Homarus americanus*, *Homarus gammarus*, and *Nephrops norvegicus*, in the crabs *Cancer magister*, *Menippe mercenaria* and *Portunus sanguinolentus*, and in the shrimp *Lepidophthalmus louisianensis*, an especially well-developed basement membrane occurs at this interface in the mesenteron-derived intestine (Yonge 1924; Johnson 1980; Factor 1981a, 1995; Trinadha Babu *et al.* 1989; Felder and Felgenhauer 1993), while there is just a modest basement membrane in the proctodeum-derived rectum of *H. americanus* and *P. sanguinolentus* (Trinadha Babu *et al.* 1989; Factor 1995). The robust basement membrane in a mesenteron-derived intestine may deter undue distension of the gut during feeding, confer a degree of elasticity upon the viscus, and/or facilitate the even distribution of mechanical forces to underlying tissues (Factor 1981a).

Bladder cells in the subepithelial connective tissue may deter distension of the intestine of *P. clarkii*. Vacuolate cells discovered by Winlow and Laverack (1972c) in the sixth abdominal ganglion of *Homarus gammarus* may be comparable to the intestinal bladder cells. In the ganglion, the vacuolate cells have been likened to 'shock absorbers' that prevent damage to (or unwanted activation of) constituent neurones.

The hindguts of *Homarus americanus*, *Homarus gammarus*, *Cancer magister*, *Callinectes sapidus*, *Portunus sanguinolentus* and *Lepidophthalmus louisianensis* exhibit a cuticle on the apical surface of the adluminal epithelium. This cuticle affords protection to soft tissues from abrasion by the luminal contents, and it may play roles in packaging faeces and conferring elasticity (Factor 1979, 1995; Mykles 1979; Johnson 1980; Trinadha Babu *et al.* 1989; Felder and Felgenhauer 1993). The cuticles in decapod crustaceans are permeable to water and salts, and they may allow the transport of both water and ions (Mary and Krishnan 1974; Malley 1977; Mykles 1979; Johnson 1980).

A cuticle is also associated with the adluminal epithelium in the intestine of *P. clarkii*. Transmission electron microscopy will be necessary to clarify if a peritrophic membrane resides over the surface mucus binding the faeces of *P. clarkii*. Peritrophic membranes are chitinous structures ostensibly deposited by midgut epithelial cells (Forster 1953; Pillai 1960; Dall 1967; Georgi 1969). Microdentition on the hindgut [rectal and (in some decapods) intestinal] cuticle, consisting of many posteriorly directed spinules, may impale the peritrophic membrane to facilitate movement of a faecal string toward the anus with each successive cycle of peristalsis (Pillai 1960; Erri Babu *et al.* 1982; Felder and Felgenhauer 1993). If a peritrophic membrane does exist around the faecal strings of *P. clarkii*, its origin from hindgut epithelial cells would seem unlikely because a cuticle segregates the adluminal epithelium from the luminal contents. Perhaps the serous

cells discovered in the subepithelial glands are involved with deposition of a peritrophic membrane. Acinar glands are especially prominent in the proximal segment of the hindgut, and the openings of their ducts are apparently restricted to sectors close to the midgut junction (Barker and Gibson 1977, 1978; Erri Babu *et al.* 1982).

Both the shapes of the adluminal epithelial cells and the appearances of the cuticular laminae vary between the tops and the apicolateral surfaces of the epithelial folds, the basolateral surfaces of the folds, and the depressed regions between the folds. Considering the cohesiveness of the cuticle at the top and apicolateral surfaces of each epithelial fold, it is doubtful that epithelial cells are subjected to significant abrasion, thus minimizing the need for extensive cell proliferation and differentiation along the basolateral surfaces. The absence of obvious mitoses by epithelial cells on basolateral surfaces of the folds is also suggestive of a low rate of cell turnover.

Two types of glandular cells in the subepithelial connective tissue of the intestine of *P. clarkii* are easy to distinguish at the light-microscopic level of resolution. The mucous cells must be implicated in the production of mucus for the packaging and lubrication of faeces and, possibly, for the elaboration of a peritrophic membrane around the luminal contents (Barker and Gibson 1978; Felder and Felgenhauer 1993). The mucous cells in the intestinal glands of *P. clarkii* resemble the cells in the hindgut (tegumental) glands of *Callinectes sapidus* and *Homarus americanus* (Johnson 1980; Factor 1995). Tegumental glands are invariably located in the connective tissue below a cuticularized epithelium (Johnson 1980; Erri Babu *et al.* 1982), although the organization of secretory units and ducts is somewhat variable (Felder and Felgenhauer 1993; Factor 1995). Functions ascribed to the secretions of tegumental glands, which might apply to those of the intestinal glands of *P. clarkii*, are the tanning of the cuticle, the deterrence of predation, and the prevention of fouling and colonization that might result from anal (rectal) drinking. Anal drinking, the intake of water from the environment by antiperistalsis, may generate the fluid pressure necessary for defecation (Fox 1952; Pillai 1960; Dall 1967).

Musculature and perimuscular connective tissue

The intestinal musculature of *P. clarkii* consists of two prominent layers of striated muscle. The propensity of cells in the inner longitudinal strips to project toward the tops of the intestinal ridges and insert on the connective tissue close to the adluminal epithelium is intriguing both morphologically and functionally. The oblique orientation of the fascicles of longitudinal muscle cells provides a plausible explanation for the peculiar wringing (torsional) form of peristalsis observed in the intestine of this crayfish (Brenner 1999). Owing to the sites of insertion, we hypothesize that bladder cells are necessary to reinforce the apices of the intestinal ridges.

Histological conclusions and ultrastructural questions

Histological features of the intestine of *Procambarus clarkii* indicate a derivation from proctodeum (ectoderm) and, thus, an affiliation with the hindgut: a cuticularized epithelium lining the lumen (see also Huxley 1880), a modest basement membrane at the interface of adluminal epithelium and subepithelial connective tissue, prominent glands in the subepithelial connective tissue, and inner longitudinal and outer circular layers of striated muscle (see also Komuro and Yamamoto 1968).

Clarification of intercellular relationships will necessitate the use of ultrathin sections for the transmission electron microscope. Do other junctions coexist with the intermediate and septate junctions already described in the adluminal epithelium (Komuro and Yamamoto 1968)? What intercellular junctions link muscle cells in the longitudinal and circular layers? Electron microscopy should be helpful in specifying the placement of neurones, as well as the neuromuscular associations, in the intestine of *P. clarkii*. Nerves near the intestinal muscle consist of large neurites enveloped by glial cells. Glial cells typically increase the conduction velocity of action potentials in crustaceans, and they are commonly found in the neural circuitry of escape responses. It is unclear why the gut should have neurones of the same variety when there is no apparent need for high conduction velocities.

We shall also want to inspect the junction of adluminal epithelium, basement membrane and subepithelial connective tissue closely in ultrathin sections to assess the claim of Komuro and Yamamoto (1968) that an 'epithelio-myonal' junction, resembling a vertebrate intercalated disk, is situated at this locale. A junction between cells of two different basic tissues is an intriguing possibility (cf. Vitzou 1882), however, it would also seem that there is ample connective tissue to construct insertions of the usual variety.

Acknowledgements

References

Barker, P. L. and Gibson, R. 1977. Observations on the feeding mechanism, structure of the gut, and digestive physiology of the European lobster *Homarus gammarus* (L.) (Decapoda: Nephropidae). – *Journal of Experimental Marine Biology and Ecology* **26**: 297–324.

Barker, P. L. and Gibson, R. 1978. Observations on the structure of the mouthparts, histology of the alimentary tract, and digestive physiology of the mud crab *Scylla serrata* (Forskål) (Decapoda: Portunidae). – *Journal of Experimental Marine Biology and Ecology* **32**: 177–196.

Brenner, T. L. 1999. The physiology of crayfish intestinal striated muscle: histology, histochemistry, and excitation-contraction coupling, MSc thesis. University of Calgary, Calgary, Alberta, Canada.

Burrage, T. G. 1978. Fine structural development and activity in the heart and midgut of the embryonic lobster, *Homarus americanus* (Milne-Edwards). PhD dissertation. Clark University, Worcester, MA.

Cavey, M. J., Wong, G. K. and Yeung, E. C. 1992. Staining of semithin glycol methacrylate sections with azure II and methylene blue. – *Transactions of the American Microscopical Society* **111**: 356–359.

Cloney, R. A. and Florey, E. 1968. Ultrastructure of cephalopod chromatophore organs. – *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **89**: 250–280.

Cole, W. H. 1941. A perfusing solution for the lobster (Homarus) heart and the effects of its constituent ions on the heart. – *Journal of General Physiology* **25**: 1–6.

Dall, W. 1967. The functional anatomy of the digestive tract of a shrimp *Metapenaeus bennettae* Racek and Dall (Crustacea: Decapoda: Penaeidae). – *Australian Journal of Zoology* **15**: 699–714.

Devi, K. N., Shyamasundari, K. and Rao, K. H. 1989. The anatomy and histology of the midgut and hindgut of *Charybdis* (*Goniohellenus*) truncata (Fabricius, 1798) (Decapoda: Brachyura). – *Folia Morphologica* **37**: 148–155.

Ebara, A. 1969. Spontaneous activity of crayfish intestine. – *Annotationes Zoologicae Japonenses* **42**: 169–175.

Erri Babu, D., Shyamasundari, K. and Rao, K. H. 1982. Studies on the digestive system of the crab *Menippe rumphii* (Fabricius) (Crustacea: Brachyura). – *Journal of Experimental Marine Biology and Ecology* **58**: 175–191.

Factor, J. R. 1979. Studies on the digestive system of the lobster, *Homarus americanus*: larval development and aspects of adult histology and ultrastructure. PhD dissertation. Cornell University, Ithaca, NY.

Factor, J. R. 1981a. Unusually complex basement membranes in the midgut of two decapod crustaceans, the stone crab (*Menippe mercenaria*) and the lobster (*Homarus americanus*). – *Anatomical Record* **200**: 253–258.

Factor, J. R. 1981b. Development and metamorphosis of the digestive system of larval lobsters, *Homarus americanus* (Decapoda, Nephropidae). – *Journal of Morphology* **169**: 225–242.

Factor, J. R. 1995. The digestive system. In Factor, J. R. (Ed.): – *Biology of the Lobster* Homarus americanus, pp. 395–440. Academic Press, San Diego.

Felder, D. L. and Felgenhauer, B. E. 1993. Morphology of the midgut–hindgut juncture in the ghost shrimp *Lepidophthalmus louisianensis* (Schmitt) (Crustacea: Decapoda: Thalassinidea). – *Acta Zoologica (Stockholm)* **74**: 263–276.

Florey, E. 1961. A new test preparation for bio-assay of factor I and gamma-aminobutyric acid. – *Journal of Physiology* **156**: 1–7.

Forster, G. R. 1953. Peritrophic membranes in the Caridea (Crustacea Decapoda). – *Journal of the Marine Biological Association of the United Kingdom* **32**: 315–318.

Fox, H. M. 1952. Anal and oral intake of water by Crustacea. – *Journal of Experimental Biology* **29**: 583–599.

Frenzel, J. 1885. Ueber den Darmkanal der Crustaceen nebst Bemerkungen zur Epithelregeneration. – *Archiv für Mikroskopische Anatomie* **25**: 137–190. plates VIII and IX.

Georgi, R. 1969. Bildung peritophischer Membranen von Decapoden. – *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **99**: 570–607.

Huxley, T. H. 1880. *The Crayfish. – An Introduction to the Study of Zoology*, D. Appleton and Company, New York.

Johnson, P. T. 1980. *Histology of the Blue Crab, Callinectes sapidus – A Model for the Decapoda*. Praeger Publishers, New York.

Komuro, T. and Yamamoto, T. 1968. Fine structure of the epithelium of the gut in the crayfish (*Procambarus clarkii*) with special reference to the cytoplasmic microtubules. – *Archivum Histologicum Japonicum* **30**: 17–32.

Lochhead, J. H. 1950. Crayfishes (and *Homarus*). In Brown, F. A. Jr (Ed.): – *Selected Invertebrate Types*, pp. 422–447. John Wiley & Sons, New York.

Malley, D. F. 1977. Salt and water balance of the spiny lobster *Panulirus argus*: the role of the gut. – *Journal of Experimental Biology* **70**: 231–245.

Mary, R. F. and Krishnan, G. 1974. On the nature and role of protein constituents of the cuticle of crustaceans in relation to permeability of the cuticle. – *Marine Biology* **25**: 299–309.

Mykles, D. L. 1977. The ultrastructure of the posterior midgut caecum of *Pachygrapsus crassipes* (Decapoda, Brachyura) adapted to low salinity. – *Tissue and Cell* **9**: 681–691.

Mykles, D. L. 1979. Ultrastructure of alimentary epithelia of lobsters, *Homarus americanus* and *H. gammarus*, and crab, *Cancer magister*. – *Zoomorphologie* **92**: 201–215.

Pillai, R. S. 1960. Studies on the shrimp *Caridina laevis* (Heller). 1. The digestive system. – *Journal of the Marine Biological Association of India* **2**: 57–74.

Shiino, S. M. 1968. Crustacea. In Kumé, M. and Dan, K. (Eds): – *Invertebrate Embryology*, Chap. 10 (Arthropoda), pp. 333–388. NOLIT Publishing House, Belgrade.

Smith, R. I. 1978. The midgut caeca and the limits of the hindgut of *Brachyura*: a clarification. – *Crustaceana* **35**: 195–205.

Talbot, P., Clark, W. H. Jr and Lawrence, A. L. 1972. Fine structure of the midgut epithelium in the developing brown shrimp, *Penaeus aztecus*. – *Journal of Morphology* **138**: 467–486.

To, T. H., Brenner, T. L., Cavey, M. J. and Wilkens, J. L. 1999.

Histology and F-actin staining of the intestine of a crayfish. – *American Zoologist* **39**: 99A [Abstract].

Trinadha Babu, B., Shyamasundari, K. and Rao, K. H. 1989. Observations on the morphology and histochemistry of the midgut and hindgut of *Portunus sanguinolentus* (Herbst) (Crustaceans: Brachyura). – *Folia Morphologica* **37**: 373–381.

Van Harreveld, A. 1936. A physiological solution for freshwater crustaceans. – *Proceedings of the Society for Experimental Biology and Medicine* **34**: 428–432.

Vitzou, A.-N. 1882. Recherches sur la structure et la formation des téguments chez les crustacés décapodes. – *Archives de Zoologie Expérimentale et Générale* **10**: 451–576. plates XXIII–XXVIII.

Vonk, H. J. 1960. Digestion and metabolism. In Waterman, T. H. (Ed.): – *The Physiology of Crustacea*, Vol. I (*Metabolism and Growth*), pp. 291–316. Academic Press, New York.

Waddy, S. L., Aiken, D. E. and de Kleijn, D. P. V. 1995. Control of growth and reproduction. In Factor, J. R. (Ed.): – *Biology of the Lobster Homarus americanus*, pp. 217–266. Academic Press, San Diego.

Winlow, W. and Laverack, M. S. 1972a. The control of hindgut motility in the lobster, *Homarus gammarus* (L.). 1. Analysis of hindgut movements and receptor activity. – *Marine Behaviour and Physiology* **1**: 1–27.

Winlow, W. and Laverack, M. S. 1972b. The control of hindgut motility in the lobster, *Homarus gammarus* (L.). 2. Motor output. – *Marine Behaviour and Physiology* **1**: 29–47.

Winlow, W. and Laverack, M. S. 1972c. The control of hindgut motility in the lobster *Homarus gammarus* (L.). 3. Structure of the sixth abdominal ganglion (6 A. G.) and associated ablation and microelectrode studies. – *Marine Behaviour and Physiology* **1**: 93–121.

Yeung, E. C. and Law, S. K. 1987. Serial sectioning techniques for a modified LKB Historesin. – *Stain Technology* **62**: 147–153.

Yonge, C. M. 1924. Studies on the comparative physiology of digestion. II. The mechanism of feeding, digestion, and assimilation in *Nephrops norvegicus*. – *Journal of Experimental Biology* **1**: 343–389.