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Unusually Complex Basement Membranes in the Midgut of Two Decapod Crustaceans, the Stone Crab *(Menippe mercenaria)* and the Lobster *(Homarus americanus)*

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ABSTRACT Ultrastructural studies of the stone crab (*Menippe mercenaria*) and the lobster (*Homarus americanus*) demonstrate that the basement membrane of the midgut (intestine) is unusually complex. In both species, the basement membrane is three-layered and has processes that form extensive networks protruding into the connective tissue. The possible functional significance of this complex structure is discussed.

The basement membrane underlying epithelial tissues is generally considered to be a continuous, electron-dense, extracellular sheet which ranges in thickness from 200 to 50,000 Å and often appears fibrous or flocculent. When viewed at low magnifications, it has been variously described in textbooks as "amorphous" (Threadgold, '76) or "homogeneous" with poorly defined inner and outer limits (Fawcett, '66). Closer examination shows that this layer may be a mat or meshwork of fine filaments embedded in an amorphous matrix (Bloom and Fawcett, '75). The basement membrane (also commonly referred to as the basal lamina or lamina densa) often appears to be separated from the epithelial cells by an intervening transparent layer (the lamina rara). The structure, function, and biochemistry of the basement membrane have recently been reviewed by Kefalides et al. ('79).

Several elaborations of this basic plan have been reported. The basement membrane of vertebrate skin may be connected to the underlying connective tissue by anchoring fibrils embedded in this tissue (Briggaman et al., '71). Additionally, Fox ('76) has shown that larger anchoring fibers may be composed of a variable number of fibrils in the skin of several aquatic vertebrates. Among the invertebrates, several authors have described unusual structural patterns for the basement membrane of the midgut in a variety of insects (Terzakis, '67; Richards and Richards, '68; Gouranton, '70; Holter, '70; Reinhardt and Hecker, '73; Hess and Pinnock, '75; Bayon and Francois, '76). These substructural variations usually include cylinders or grid-like patterns, sometimes composed of hexagonal units.

Ultrastructural studies of the connective tissue layer surrounding the digestive epithelium in two decapod crustaceans, the stone crab *Menippe mercenaria* (Brachyura: Xanthidae) and the lobster *Homarus americanus* (Macrura: Nephropidae), demonstrate that the basement membrane of the adult midgut (intestine) is unusually complex. This is the first description of a complex basement membrane in this major group of invertebrates.

MATERIALS AND METHODS

The stone crabs used in this study were trapped in the Indian River coastal lagoon of Florida and the American lobsters were obtained from commerical sources. The digestive tissues of both species were excised from living animals, fixed for light microscopy in seawater Bouin's fluid, and embedded in Paraplast. Sections were stained with Harris's hematoxylin and eosin, Mallory's triple connective tissue stain, 1% alcoholic aniline blue and picric acid–acid fuchsin, and periodic acid-Schiff's reagent (Humason, '62). Tissues were fixed for electron microscopy in 3% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7.0. The buffer contained 30 mg/ml NaCl and 20 μg/ml $CaCl_2$. Fixation was carried out at 6°C for 3–6 hours and was followed by 5 washes in solutions of buffer with decreasing concentrations of salts. Speci-

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mens were post-fixed in 1.0% OsO_4 in buffer for 5 hours, treated with 2% uranyl acetate, dehydrated in ethanol which was replaced by propylene oxide, and embedded in a mixture of Epon 812–Araldite 506. Thin sections were stained with uranyl acetate and lead citrate.

OBSERVATIONS

The basement membrane of the midgut of the lobster is illustrated in Figures 1–3. When viewed with the light microscope (Fig. 1) it can be seen to be a wavy structure which follows the folding of the simple, columnar epithelium of the midgut and separates it from the underlying connective tissue. The basement membrane stains positively with PAS and aniline blue and is slightly eosinophilic. It varies in thickness from 0.2 to 0.4 μm and can be seen electron-microscopically to be pierced by branching channels (Figs. 2 and 3). The central layer consists of a finely granular, extremely uniform material and is coated on both sides with thin, fibrous, relatively electron-dense outer layers (Fig. 3). In addition, structures which range from roughly circular to elongate in section lie adjacent to the basement membrane proper and can be seen to consist of the same finely granular material coated with a similar electron-dense outer layer. Serial sections indicate that the larger of these extensions, at least, are processes that extend from the basement membrane. The fibrous outer layer coating the basement membrane and its processes is drawn out into strands which bridge the channels in the membrane and which also interconnect its processes (Fig. 3). The electron-dense strands, together with the processes of the basement membrane, form an extensive network (approximately 0.4 μm thick) along the connective tissue surface of the basement membrane (Fig. 2). Processes of fibroblasts are commonly associated with this network (Fig. 3). A similar network is only intermittently present on the surface of the basement membrane which faces the epithelium.

The basement membrane of the midgut of the stone crab is illustrated in Figures 4–6. It is considerably thicker than that of the lobster (approximately 1.5 μm) and appears to have two layers when viewed with the light microscope (Fig. 4). Its staining characteristics are similar to the basement membrane in the midgut of the lobster – it is strongly PAS-positive, strongly aniline blue-positive, and slightly eosinophilic. When observed with the electron microscope, the basement membrane is three-layered and does not appear to have channels (Fig. 5). The central layer has a coarse, reticulated appearance and is coated on both sides by outer layers of fibrous material (Fig. 6). The outer layer facing the epithelium is a simple, thin, electron-dense sheet approximately 0.06 μm thick. The outer layer on the connective-tissue side of the basement membrane is generally thicker (approximately 0.2 μm) and relatively less electron-dense. In addition, extensions of this layer protrude into the connective tissue. These extensions are often T-shaped in transverse section and the fibrous material is commonly drawn out into discrete, banded fibers at the indistinct boundary between this layer and the general matrix of connective tissue (Fig. 6). Extensions of the fibrous layer are especially numerous and prominent where the basement membrane folds toward the lumen of the gut to follow the longitudinal infoldings of the midgut epithelium. In these areas they form a network that extends approximately 1–2 μm into the connective tissue (Fig. 5). Fibroblasts are usually associated with this fibrous outer layer and its extensions (Fig. 6). The basement membranes in the midgut of both lobsters and stone crabs were often separated from the epithelium (Fig. 4), presumably as a result of mechanical damage during preparation, but were not separated from the connective tissue.

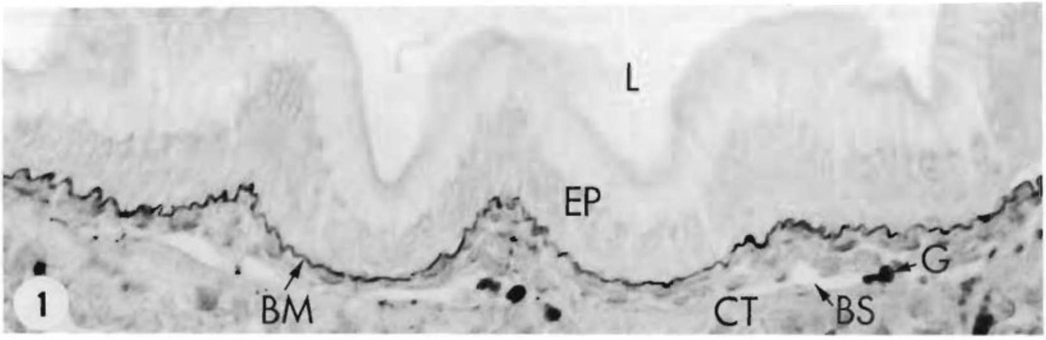
Abbreviations

BM, Basement membrane
BS, Blood sinus
CL, Central layer of basement membrane
CT, Connective tissue
EP, Epithelium
F, Fibroblast
G, Granulocyte
L, Lumen of midgut
OL, Outer layer of basement membrane

Fig. 1. Basement membrane underlying the midgut epithelium of the lobster. Transverse section stained with 1% aniline blue (alc.), counterstained with picric acid–acid fuchsin. × 270.

Fig. 2. Basement membrane (lobster) showing channel (arrowhead) and network of processes on connective tissue surface. × 9400.

Fig. 3. Portion of the basement membrane (lobster) showing a branching channel, the central layer and fibrous outer layers, processes of the basement membrane (large arrowheads), strands which interconnect processes and bridge channels (small arrowheads), processes of fibroblasts, processes of epithelial cells, and connective tissue. × 46,000.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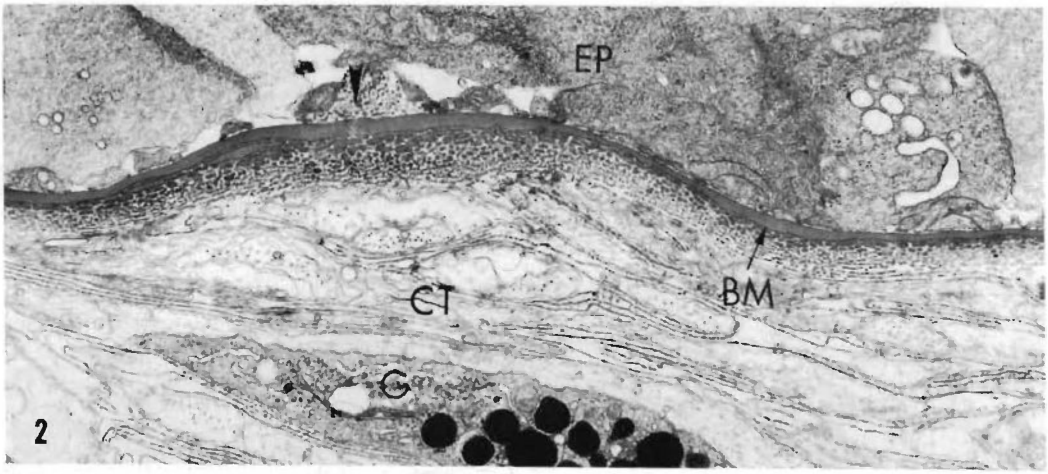
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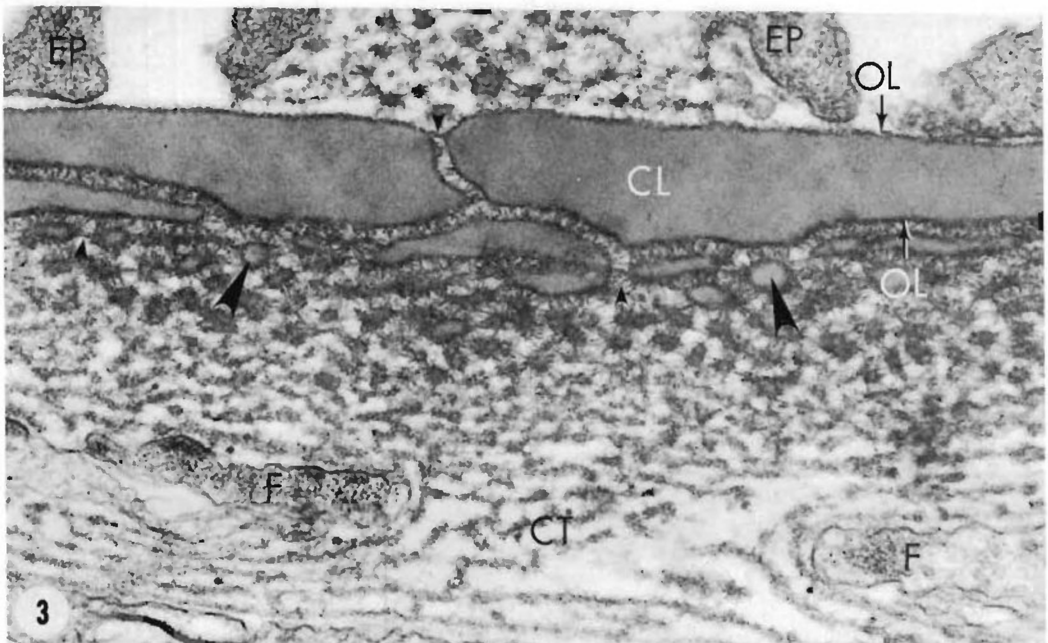
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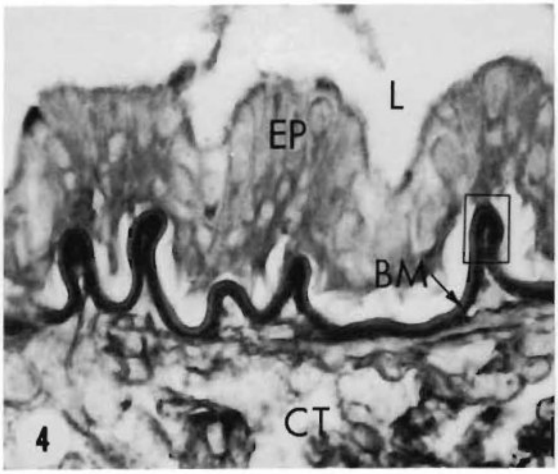
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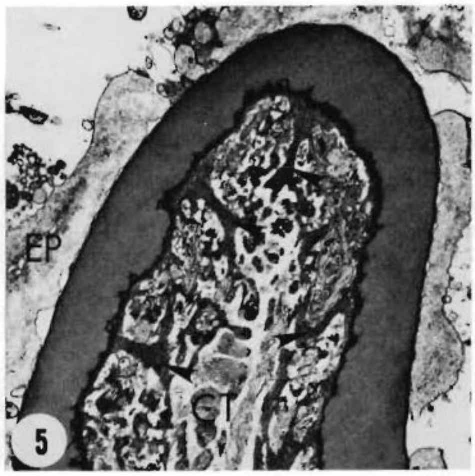
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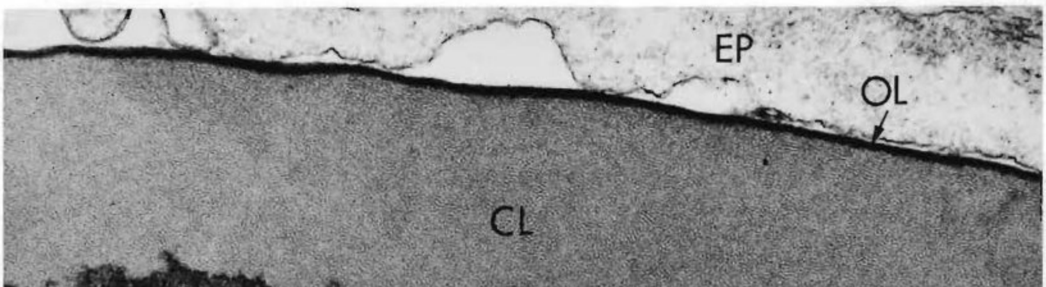
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DISCUSSION

The use of the term "basement membrane" to describe the structures in the midgut of the lobster and stone crab may require some justification, particularly in light of the complex nature of these structures. "Basement membrane" has been used in a variety of ways. Kefalides et al. ('79) point out that the term was coined in 1857 by Robert Todd and William Bowman to describe the extracellular subepithelial layer seen with the light microscope in a variety of tissues. Ultrastructural observations have shown that in some instances the basement membrane described from light microscopy is actually a complex of structures that can be distinguished with the electron microscope, and includes the reticular layer, which is now considered to be distinct from the basement membrane. Minor et al. ('76) state, "Microscopically a basement membrane is recognized as a well-defined lamina of extracellular matrix associated with the surface of a variety of cells, including epithelial, mesothelial, endothelial, and muscle cells." Kefalides et al. ('79) use the term to refer to the "dense, amorphous matrix observed in the electron microscope as lying between the lamina lucida and the fibrillar [reticular] layer . . . just below the basal layer of the epidermis," and for the layer underlying the urinary, reproductive, respiratory, and digestive epithelia. They also use it to describe the extracellular matrix of the glomerular capillary, renal tubule, capsule of the ocular lens, Descemet's membrane of the cornea, Reichert's membrane of the rodent parietal yolk sac, and the layer surrounding Schwann cells, muscle cells, and adipocytes. The term has been applied, then, to extracellular layers with a variety of structural plans ranging from the typical, one-layered, electron-dense lamina with poorly defined inner and outer limits, which underlies many epithelia, to the three-layered glomerular capillary basement membrane, in which the inner and outer layers are continuous with the surface coats of the surrounding cells (Latta and Johnston, '76). Although the basement membrane is often described as "amorphous," substructure in the form of oriented fibrils (Jollie, '68; Clark et al., '75) or hexagonal patterns (Jakus, '64) has been reported, in addition to the reports of grid-like patterns in insect basement membranes cited above.

Biochemically, basement membranes consist of a collagenous component, shown in several instances to be a unique type of collagen molecule (type IV), and a non-collagenous component, possibly including glycoproteins and glycosaminoglycans (reviewed by Kefalides et al., '79).

The basement membranes in the midgut of the lobster and stone crab appear to fit into the broad morphological definition of "basement membrane" as an extracellular, electron-dense matrix forming an extensive layer between the basal surface of the digestive epithelium and the underlying connective tissue. Additionally, the strongly positive PAS-reaction, common to many basement membranes, indicates the presence of carbohydrates possibly associated with the non-collagenous component. Although they fit the general definition, the basement membranes in the midgut of the lobster and stone crab are more complex than the typical basement membrane in several respects: (1) they have three layers—a thick central layer which is coated on both sides with thin fibrous outer layers; (2) they lack a substantial electron-transparent zone (lamina rara); (3) the central layer does not appear amorphous in the stone crab, although it does in the lobster; (4) the basement membrane proper (i.e., the basement membrane excluding any processes or extensions) has well-defined limits; and (5) extensions of the basement membranes protrude into the connective tissue.

Perhaps the most interesting features present are the processes of the basement membrane of the lobster and the extensions of the outer layer of the basement membrane of the stone crab. Both are variations of the same structural theme and form extensive networks which appear to connect the basement membrane and the connective tissue in this region of transition; they may represent complex versions of the anchoring fibrils and fibers found in the skin of vertebrates. Although the midgut epithelium was often artifactually separated from the basement membrane, the

Fig. 4. Basement membrane underlying the midgut epithelium of the stone crab. Note artifactual separation of epithelium from basement membrane. Transverse section stained with PAS. × 465.

Fig. 5. Fold in the basement membrane (stone crab), similar to the area in the box in Figure 4, showing the network of extensions of the fibrous layer (arrowheads) into the connective tissue. × 5200.

Fig. 6. Portion of the basement membrane (stone crab) illustrating the central and fibrous outer layers, extensions of the outer layer on the connective tissue surface (large arrowhead), fibers at the edge of the extension (small arrowheads), and processes of fibroblasts. × 22,100.

basement membrane was never observed to be separated from the surrounding connective tissue. This observation provides indirect evidence for a possible anchoring function of the processes and extensions of the basement membrane.

The midgut of arthropods is distinct from the foregut and hindgut both developmentally and structurally. The foregut (esophagus and stomach) and hindgut (rectum) are lined with a thin layer of cuticle which likely provides structural reinforcement for the underlying tissues and may provide elasticity when the wall of the gut is distended during feeding. The midgut has no such cuticular lining and it is interesting to note that this complex basement membrane has been observed only in the midgut of the lobster and stone crab; preliminary observations indicate the presence of a typical basement membrane in other regions of the gut. Complexities of the basement membrane in various insects are also reported to be present in the midgut. It seems plausible that the complex basement membranes in the midgut of the stone crab and the lobster may serve to reinforce the midgut against dilation during feeding, provide elasticity, or evenly distribute the mechanical forces to underlying tissues.

The basement membrane in the midgut of the stone crab is unusually thick and the central layer can be distinguished from the network of extensions with the light microscope. This feature suggests it would be especially suitable for experimental studies on the origin and development of the basement membrane using autoradiography at the light microscope level.

Demonstrating incontrovertibly that the structures that underlie the digestive epithelium in the midgut of the lobster and stone crab are basement membranes must await detailed biochemical characterization beyond the scope of this report, including the demonstration of type IV collagen. Morphological evidence alone, however, suggests that they are some of the more complex versions of the basement membrane yet to be reported.

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