Fine Structure at the Basal Surface of Intestinal Epithelium in the Midgut Region of the Balanidae, with Special Reference to "Neural-like" Processes

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ABSTRACT Observations on fine structure at the basal end of the intestinal epithelium in the midgut region of *Balanus balanoides* and *Balanus improvisus* reveal complex interrelationships among several tissues. Numerous elongate cell processes extend towards the intestinal epithelium penetrating between layers of intestinal muscle through blood spaces and into the basal lamina underlying the epithelium.

Two types of morphological relationships occur between cell processes and the basal end of the intestinal epithelial cell: 1. The cell process may penetrate the basal lamina and lie closely apposed to the epithelium. 2. The cell process may give rise to narrow, medially-directed, finger-like extensions (projections). The narrow projections penetrate the basal lamina and, in addition, terminate as dilated bulbs within inpocketings of the epithelium. In some respects the cell processes are suggestive of neural tissue.

Barnacles, an especially well-adapted group of crustaceans, are found throughout the world (Newman et al., '69). Their ecological distribution and taxonomic position have been studied intensely (Newman et al., '69), however, little is known about the mechanisms that enable these organisms to function so successfully in their selected niches.

During studies on fine structure of the midgut in *Balanus balanoides* and *B. improvisus* complex relationships were observed among several tissues and structures at the basal lamina of the intestinal epithelium.

This paper describes some of these relationships. The tissues and structures studied include: 1. Basal surface of the intestinal epithelial cells. 2. The basal lamina of the intestinal epithelium. 3. A system of open blood spaces. 4. Numerous elongated cell processes.

Observations on the cell processes reveal that they extend through the blood spaces, penetrate the basal lamina and terminate on the basal surface of the intestinal epithelium. Their fine structure is suggestive of neural tissue. The relationship between the cell processes and intestinal epithelium is of special interest and will be emphasized.

MATERIALS AND METHODS

The two species used in this study, *Balanus balanoides* and *B. improvisus* were collected in waters around Staten Island, New York. They were maintained in continuously aerated artificial sea water and fed newly hatched Artemia larvae. Approximately six specimens of each species were selected for microscopic examination. They were quickly removed from their outer calcareous shell, flooded with fixative and cut into smaller pieces on an ice-cooled platform.

For observations with the light microscope tissues were fixed in cold 10% neutral formalin or a cold glacial acetic acid-ethanol mixture, 1:3. Paraffin sections of appropriate thickness were used for observations on glycogen distribution and general morphology. The P.A.S. reaction with and without amylase was used to identify glycogen (Barka and Anderson, '65). Sec-

tions stained with Azure-B or H. and E. were used for general morphology.

For electron microscopic observations, small pieces of tissue were fixed in cold 3% glutaraldehyde buffered with sodium cacodylate to pH 7.2 to 7.4 for one-and-one-half hours. Tissues were washed in cold buffer, and postfixed in 1% osmium tetroxide buffered with cacodylate to pH 7.2 and embedded in epon according to established procedures (Luft, '61; Koulish, '69). Thicker (2 μ) sections were either stained with toluidine blue (Bennett and Radimska, '66) or used unstained with phase microscopy for observations on general morphology. Thin sections were stained in either a 5% solution of uranyl acetate in methanol (Koulish, '69) or lead citrate (Venable and Coggeshall, '65) and collected on uncoated grids and viewed at 50 Kv in a Hitachi HS7S electron microscope.

OBSERVATIONS

The midgut of the U-shaped alimentary canal in the Balanidae contains a single layer of epithelial cells. For purposes of orientation, a brief description of these cells follows. A detailed account will be presented elsewhere.

The luminal surface of the intestinal epithelial cell ends in numerous closely spaced, orderly arranged microvilli. A labyrinthine system of infoldings characterizes the basement membrane. The infoldings may extend apically for almost half the length of the cell or turn back towards the basal surface where they appear to "undercut" segments of basal cytoplasm (fig. 1). The nucleus and Golgi elements tend to be located in the basal half of the cell. Mitochondria and lysosome-like bodies are distributed throughout the cell. Ribosomes are abundant and distributed both freely in the cytoplasm and attached to vesicular or short lengths of cisternal types of endoplasmic reticulum. Microtubules are aligned parallel to the long axis of the cell and appear to be more numerous in its basal end.

In addition to these organelles, the intestinal epithelial cells contain several types of distinctive membrane-bound granules or vesicles. These structures differ for the two species studied and may reflect differences between the species and/or changes in the physiologic state of the organism at the time they were sacrificed. Observations are in progress which should clarify this point.

Of the several types of vesicles present in *B. balanoides*, two of them accumulate at the basal end of the cell and will be described in this paper. Only one of the two, a heteromorphic vesicle, was also observed in the second species, *B. improvisus*.

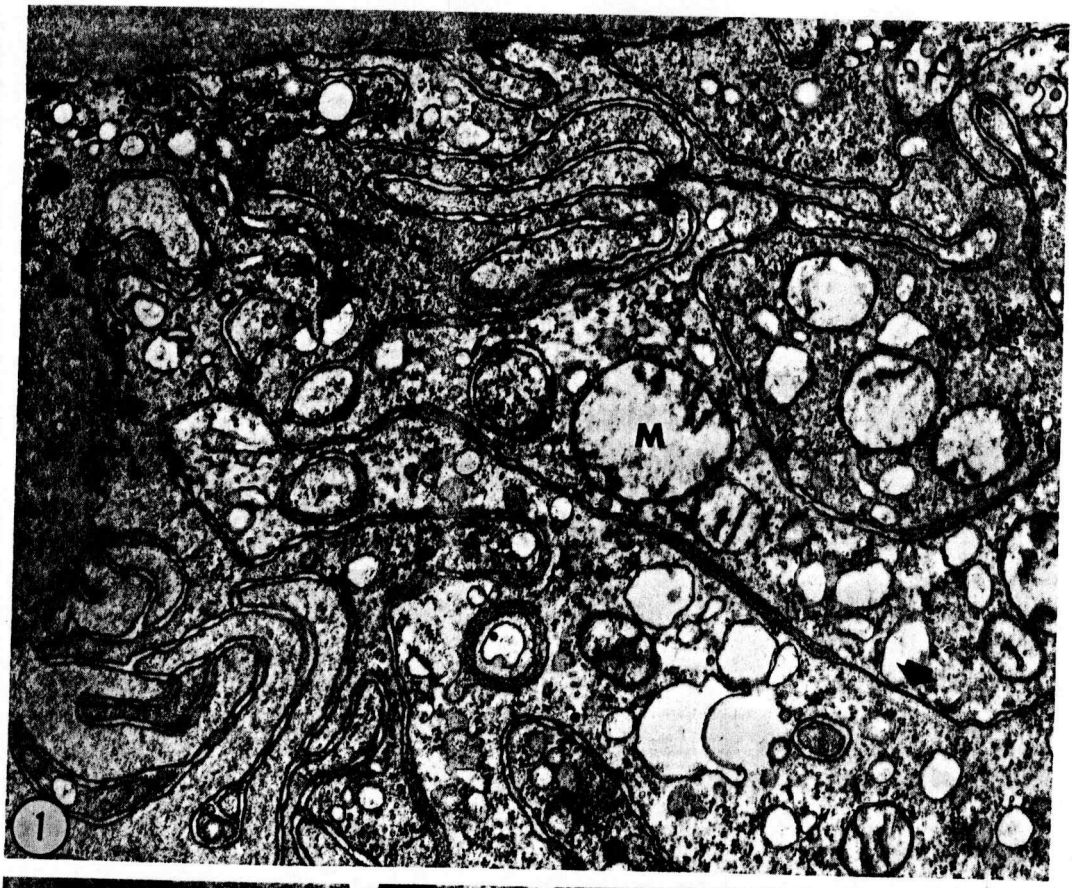
The first of these structures, the electron opaque granule (EOG) is generally circular or oval in section. They may also assume "S" and "U" shapes which suggest that they are somewhat plastic in nature (fig. 2). Dimensions of the EOG are approximately 0.4 μ x 0.3 μ x 0.15 μ.

Many myelin-like membrane bodies are interspersed among the EOGs. The myelin-like bodies vary widely with respect to density, from those with distinct concentric membrane structure against an electron-lucent background to those whose membranes are barely discernible against an electron-opaque background (fig. 3). These observations lead to the tentative conclusion that the EOG and myelin-like body are related, i.e., the one is gradually transformed into the other.

The second type of structure (observed in both species) is heteromorphic and consists of an electron dense core, a less dense ring and an outer membrane (fig. 4, 6). It ranges in size from 500 to 1,000 Å. Groups of these vesicles are confined to the basal end of the cell and usually appear to be "segregated" from the remainder of the cell

Abbreviations

L, Basal lamina
S, Basal surface of epithelium
B, Blood space
P, Cell process
O, Electron-opaque vesicles
EB, End-bulb of projection
H, Heteromorphic vesicles
E, Intestinal epithelial cell
I, Intestinal muscle
MT, Microtubules
M, Mitochondria
MB, Myelin body
PR, Projection



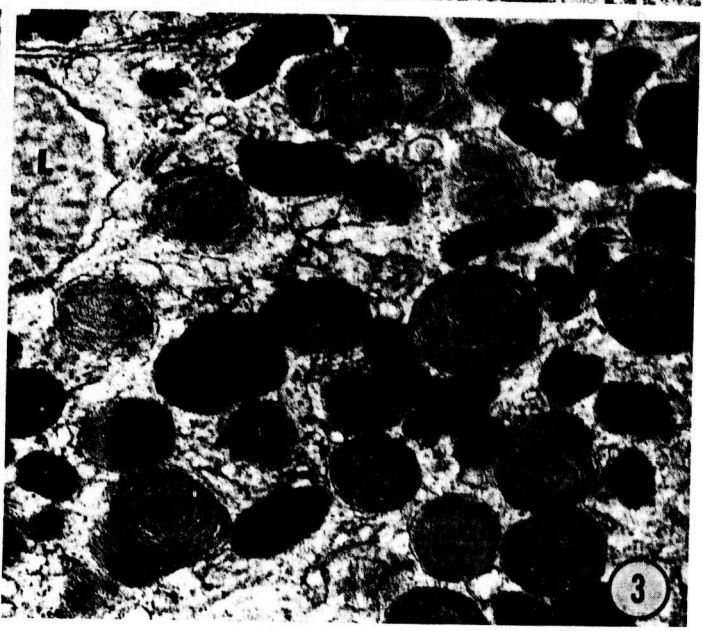


Fig. 1 Basal end of intestinal epithelium. Note complex infoldings of surface membrane, ribosomes, microtubules (mostly X-sections), gap junction (dense arrow). *B. balanoides*. Lead stain. × 15,000.

Fig. 2 Basal end of intestinal epithelium cell. Note assorted shapes of electron-opaque granules (EOG). *B. balanoides*. Lead stain. × 15,000.

Fig. 3 Basal end of intestinal epithelial cell containing myelin-like bodies interspersed with EOGs. Note differences in density of myelin-like bodies. *B. balanoides*. Lead stain. × 25,000.

by an infolding of the basal surface. Some heteromorphic vesicles may lack either the core, peripheral ring or both of these components leaving an "empty" vesicle. Microtubules are often observed in the "segregated" area and, in general, they are aligned along a different axis than that in the surrounding cytoplasm (figs. 4, 5, 6).

Portions of cell processes, also containing microtubules, may lie closely apposed to the basal surface of the intestinal epithelial cell in some areas (fig. 11). A question arises as to whether the "segregated" area is part of the epithelial cell or results from complex interrelationships between cell processes and the basal surface of the epithelial cell. (See discussion section, below.)

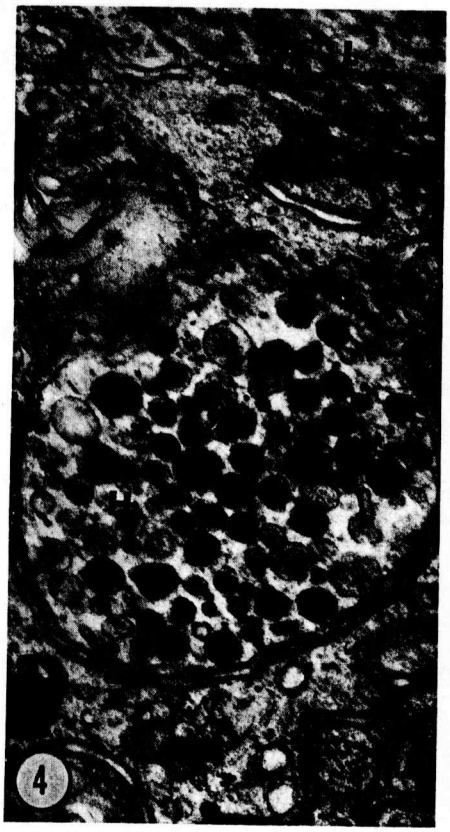
Underlying the basal end of the intestinal epithelium is a non-cellular layer approximately 1 to 2 μ thick, the basal lamina. Electron microscopic observations show this lamina to be "sponge-like" in appearance consisting of irregularly-shaped light and dark trabeculae (fig. 7). Profiles of numerous membrane bound projections penetrate the basal lamina and extend towards the basal end of the intestinal epithelial cell. These projections will be described in more detail below. An extensive system of open blood spaces and/or channels envelop the midgut and appear to be continuous with the basal lamina (figs. 7, 8). Although it is questionable practice to impart dynamic interpretation to static micrographs, there is the impression that the trabeculae of the basal lamina "diffuse" or "expand" ino the blood spaces thereby contributing some material to its contents. The blood channels extend along the length of the gut and also branch laterally towards the body wall. In their passage peripherally, the channels become narrower and wind irregularly between tissues and between cells of a tissue. Peripheral to the basal lamina but not necessarily contiguous with it, are thin layers of longitudinally and transversely arranged muscle. Numerous elongated cell processes extend medially between the muscle layers, penetrate the blood channels and continue toward the basal lamina of intestinal epithelium (figs. 7, 8, 13). The relationships among several of the structures described above are evident in figure 7.

It is difficult to identify the zone containing the cell processes in the light microscope with routine stains. However, the PAS stain and amylase digestion provide indirect evidence for this zone. These reactions reveal extensive glycogen deposits in tissue surrounding the intestine *except* for a narrow zone immediately adjacent to the intestine. Electron microscopic examination of this narrow glycogen-free zone shows it to contain the elongated cell processes extending toward the intestine.

The cell processes contain numerous microtubules often equidistantly spaced. Depending upon species, they may, in addition, contain varying quantities of: mitochondria, multivesicular bodies and heteromorphic vesicles. The heteromorphic vesicles contain cores of varying electron density and, in general, less dense peripheral rings (figs. 12, 13). They are similar in appearance to the bodies observed in the basal end of intestinal epithelial cells (figs. 4, 5, 6).

The heteromorphic vesicles, more abundant in the processes of *B. balanoides* than in *B. improvisus*, tend to aggregate in the distal segment of the cell processes, where they (the processes) approach the basal lamina of the intestinal epithelium (figs. 12, 13). At the juncture between cell process and basal lamina narrow, finger-like projections extend from the process, penetrate the basal lamina and finally terminate on the basal end of the epithelial cell (figs. 8, 13, 14, 15). The projections vary in length and are approximately 400 to 800 Å wide. They may contain a microtubule and/or some medium electron-dense material. Irregularly-shaped broader projections, containing heteromorphic vesicles, are also observed in the basal lamina (fig. 13).

The extent of the projection in the basal lamina and its terminalization on the basal surface of the intestinal epithelium may be observed in sections cut parallel to its (the projection's) long axis. In *B. improvisus* the projection ends as a slightly widened bulb inserted into an inpocketing of the basement membrane (figs. 14, 15, 16). Between the end-bulb of the projection and the inpocketing of the basement membrane, a narrow cleft, approximately 200 Å in width, containing a medium elec-



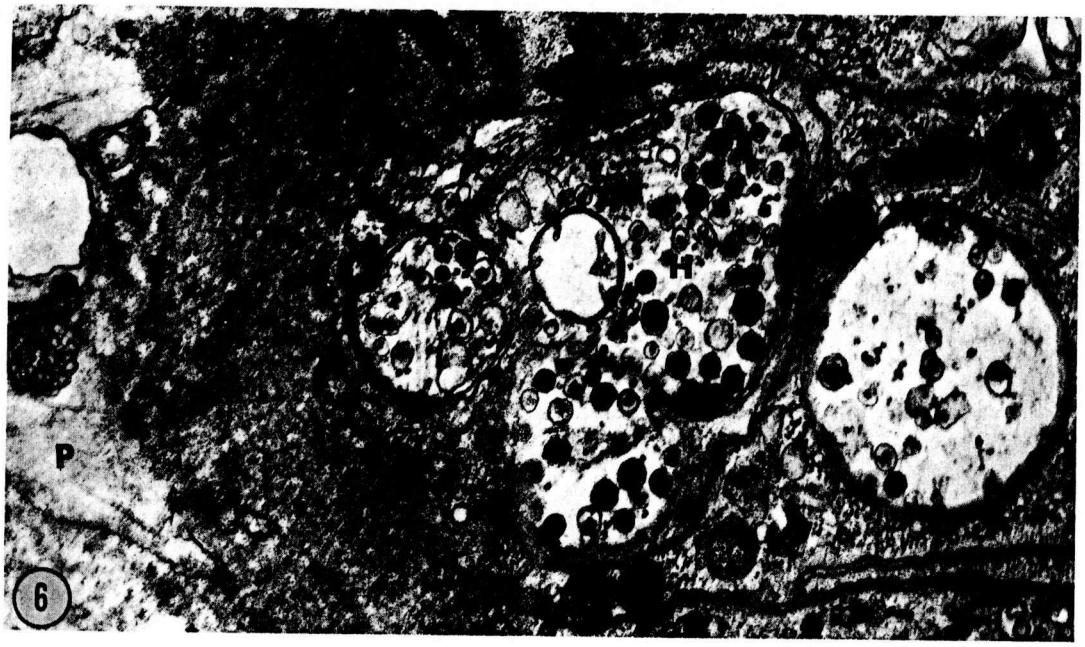
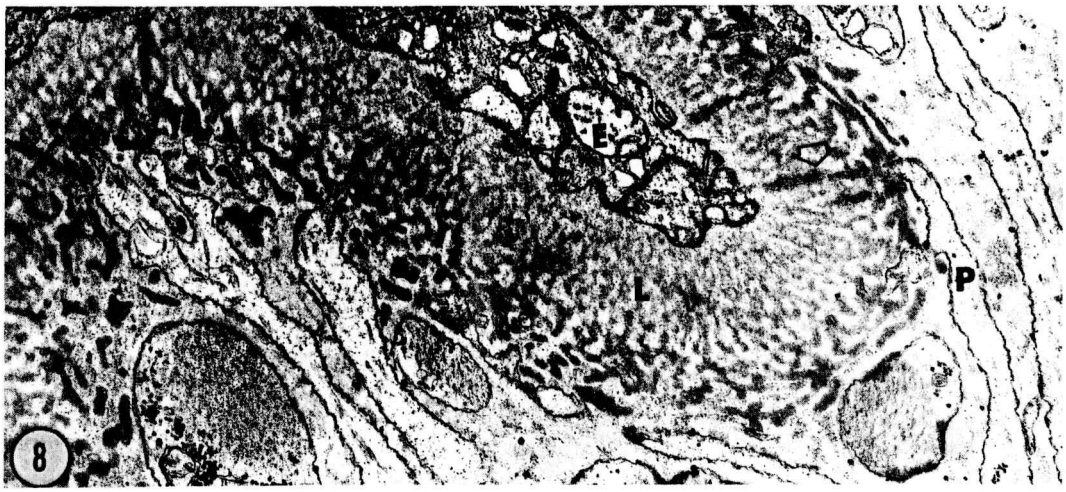


Fig. 4 Basal end of intestinal epithelial cell. Note "segregated area" confined by double membrane and containing numerous heteromorphic vesicles. *B. balanoides*. Lead stain. × 27,500.

Fig. 5 "Segregated area" in basal end of epithelial cell. Note electron-lucent background and few heteromorphic vesicles. *B. balanoides*. Lead stain. × 20,000.

Fig. 6 Several "segregated areas" at basal end of intestinal epithelial cell. Note differences in number of heteromorphic vesicles in these areas, microtubules in each area aligned along different axis and electron-lucent background. Also note portion of cell process (p) distal to basal lamina. *B. balanoides*. Lead stain. × 20,000.



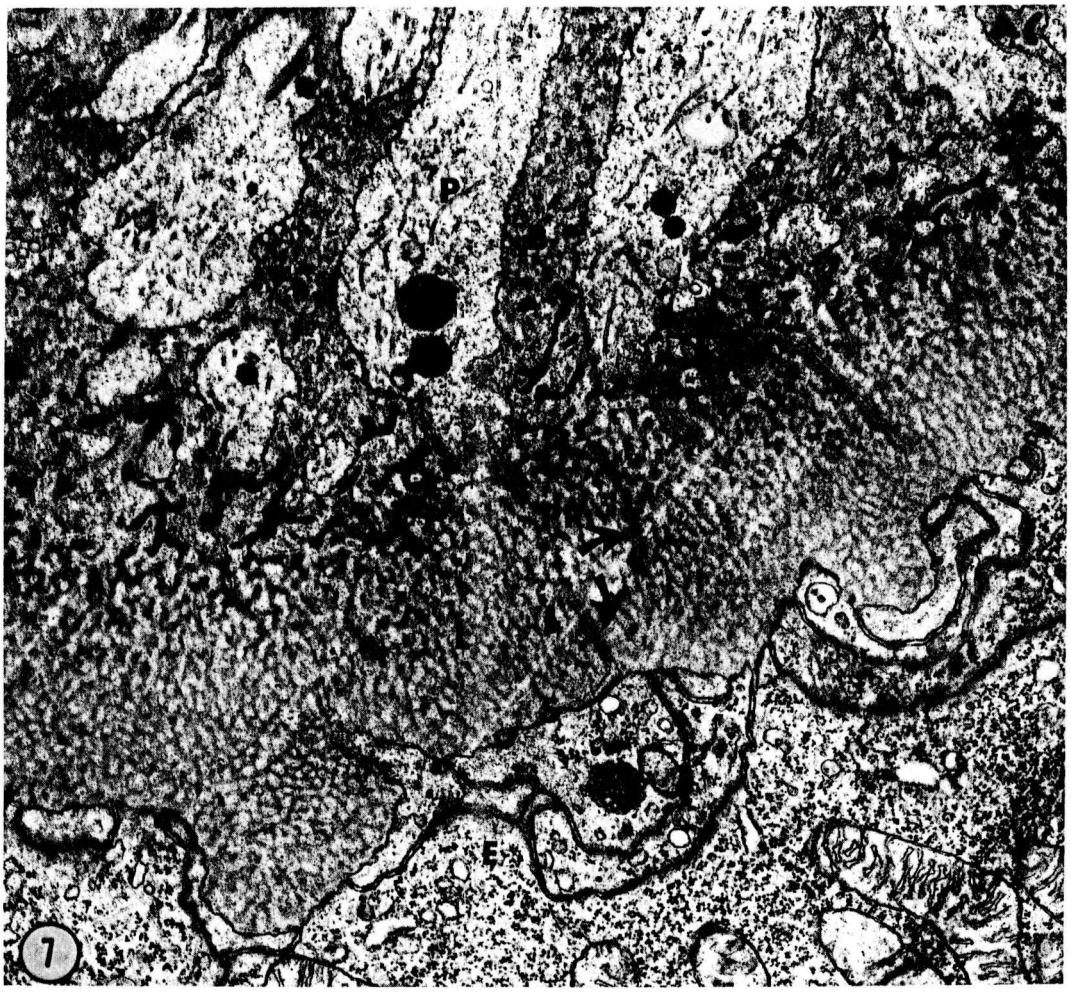


Fig. 7 Basal surface of intestinal epithelium. Note "trabeculae" nature of basal lamina, cell processes containing microtubules, projections (PR) from cell processes extending through basal lamina, blood spaces between processes and apparently continuous with basal lamina. *B. improvisus*. Lead stain. × 13,500.

Fig. 8 Region at basal surface of intestinal epithelium. Note narrow projections extending from cell processes. Projections penetrate basal lamina (open arrow). *B. improvisus*. Lead stain. × 13,500.

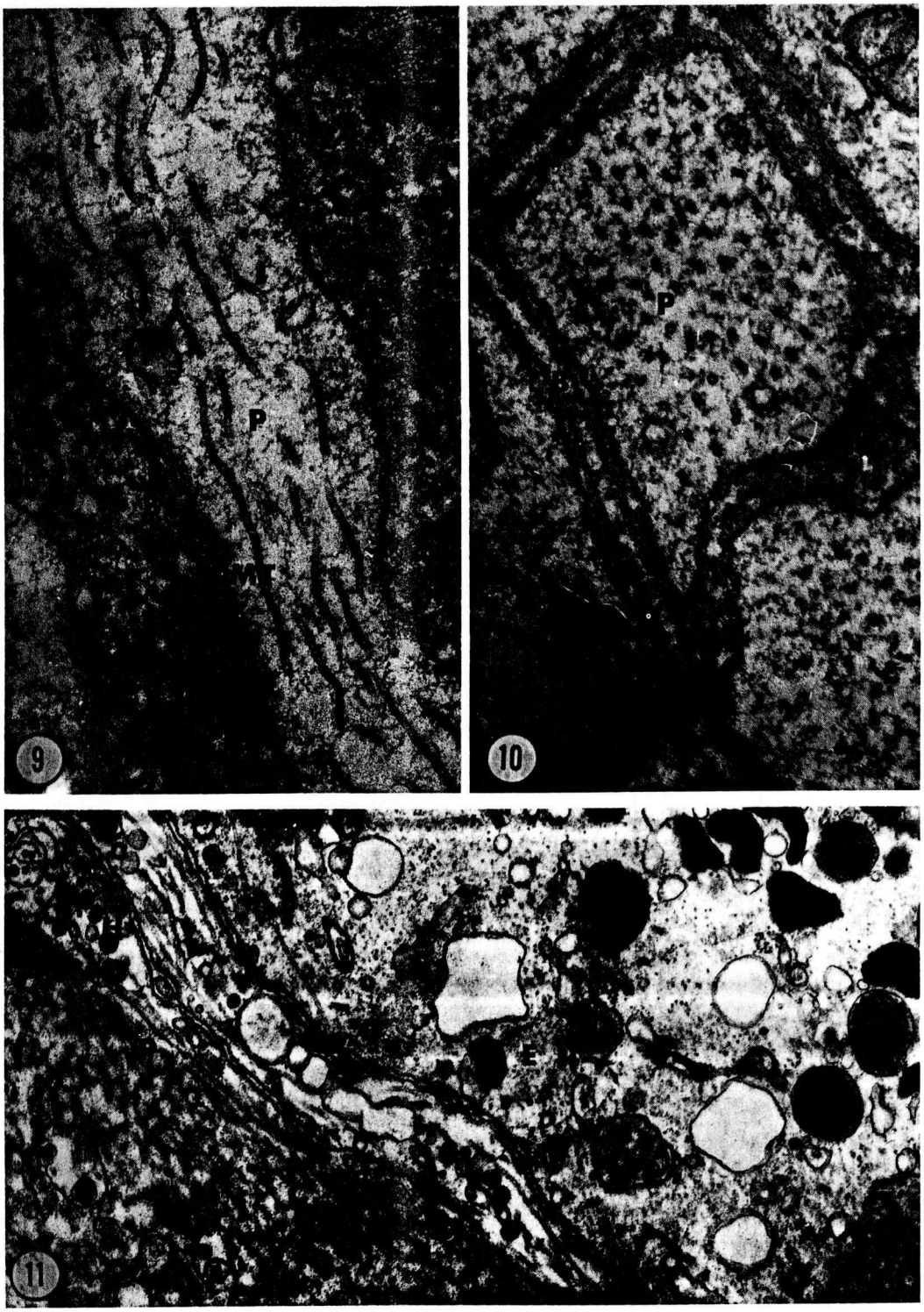
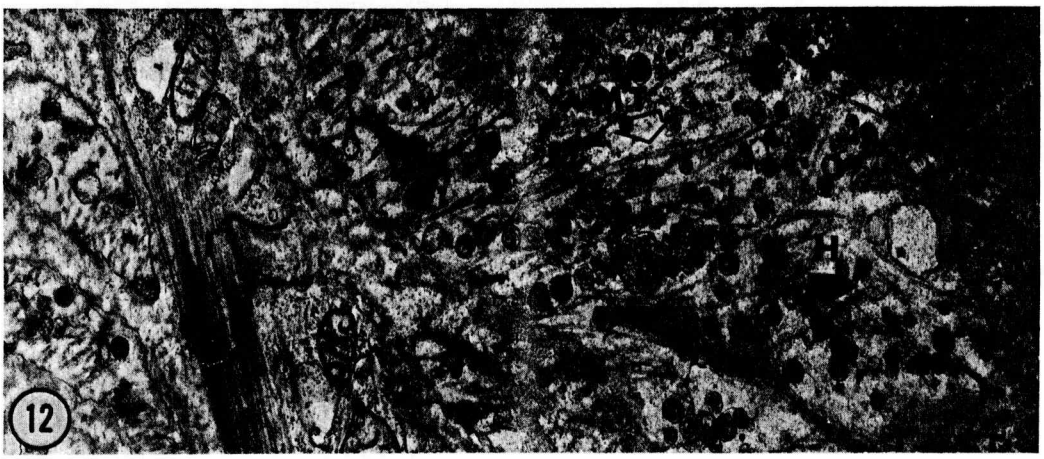


Fig. 9 Portion of cell process, longitudinal section. Note microtubules approximately equidistantly spaced. *B. improvisus*. Uranyl stain. × 37,500.

Fig. 10 Cross-section through cell processes. Note equidistantly spaced microtubules, mitochondrion in one process and blood spaces surrounding cell processes. *B. improvisus*. Uranyl stain. × 37,000.

Fig. 11 Cell processes containing heteromorphic vesicles and microtubules closely apposed to basal surface of intestinal epithelial cell and beneath basal lamina. *B. balanoides*. Lead stain. × 20,000.



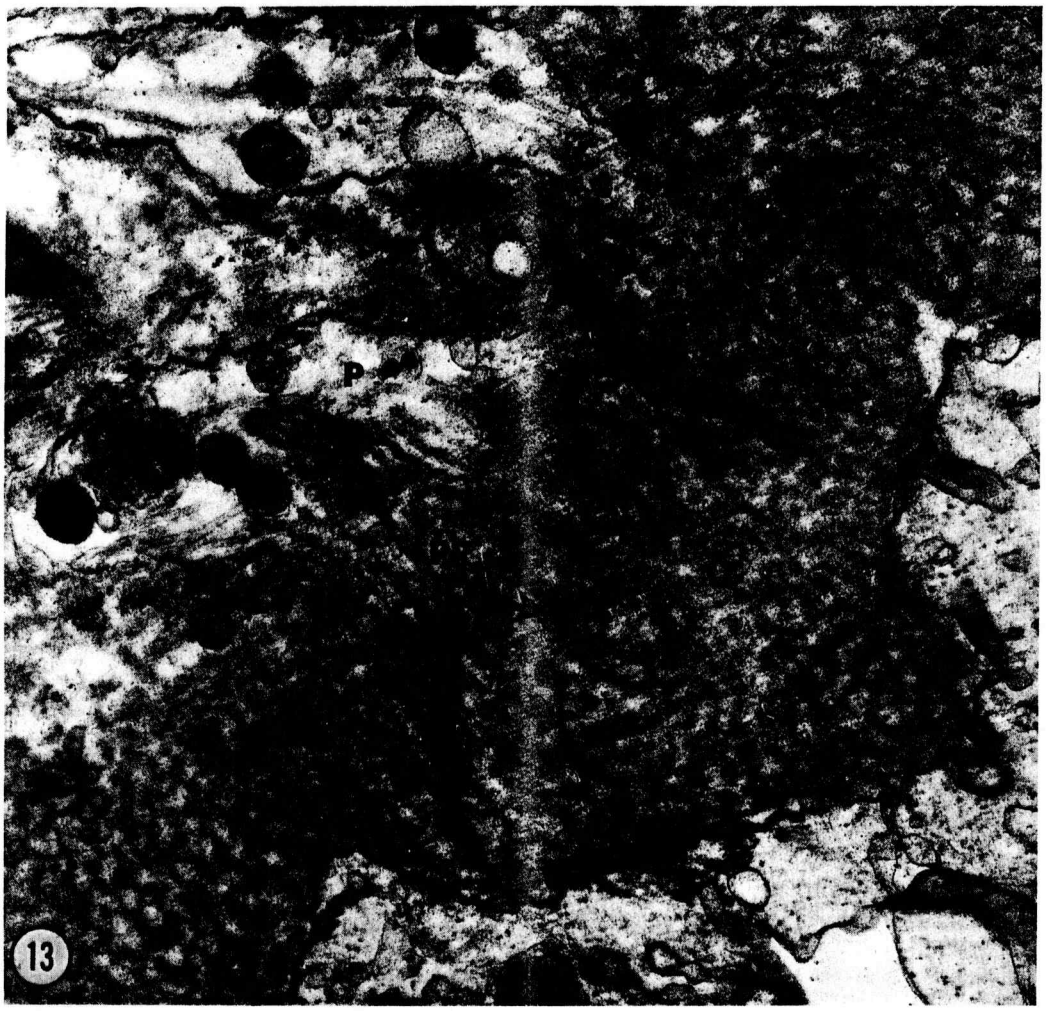
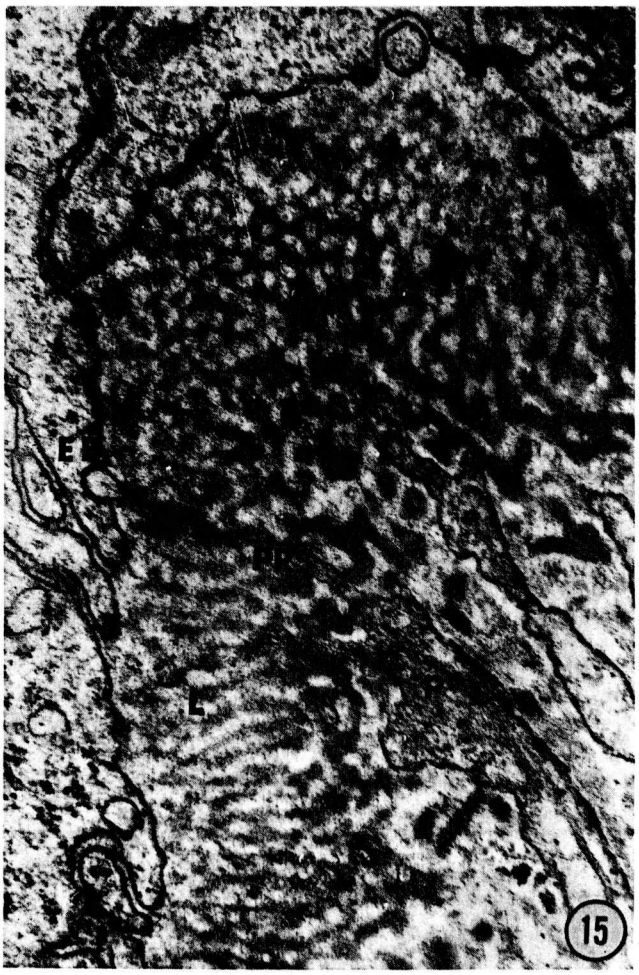


Fig. 12 Cell processes at juncture with basal lamina. Note numerous microtubules (open arrow) and heteromorphic vesicles (dense arrow) within processes. *B. balanoides*. Lead stain. × 13,000.

Fig. 13 Cell processes at juncture with basal lamina. Note projections (PR) extending from processes and penetrating basal lamina. Heteromorphic vesicles and microtubules are in processes. *B. balanoides*. Lead stain. × 27,500.



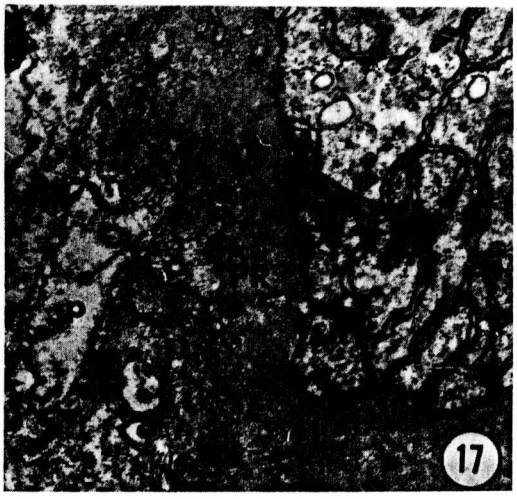


Fig. 14 Narrow projection extending from process and terminating as dilated bulb within inpocketing of surface of intestinal epithelium. Note microtubule within projection. *B. improvisus*. Lead stain. × 37,500.

Fig. 15 Narrow projection extending from process and terminating within inpocketing of epithelial surface. *B. improvisus*. Lead stain. × 30,250.

Fig. 16 Narrow projection penetrating through basal lamina tapering to a point within inpocketing of surface of intestinal epithelium. *B. balanoides*. Lead stain. × 27,500.

Fig. 17 Narrow projection penetrating basal lamina and terminating as dilated bulb within surface of intestinal epithelium. *B. balanoides*. Lead stain. × 15,000.

tron-dense material, is observed. The contour of the membranes on both sides of the cleft (as well as the distance between the membranes) is very regular (figs. 14, 15). In *B. balanoides* projections occasionally appear to terminate as points rather than as end-bulbs (fig. 17). Additional observations on the relationship between the terminal structure of the projection and the basal surface are in progress.

DISCUSSION

Although physiological data for transport across the basal surface of intestinal epithelium in the Balanidae is lacking, the following morphological observations indicate that transport is probably commonplace. (1) The basal surface is thrown into numerous, complex infoldings thereby increasing surface area and opportunity for transport. Correlation of basal surface infolding with transport has been established for several tissues (Pease, '56; Anderson and Harvey, '66). (2) The basal lamina of the intestinal epithelium, in this region, is apparently contiguous with an open system of blood spaces. The continuous bathing of this membrane with body fluids probably maximizes opportunities for transport of substances across its surface.

Numerous cell processes have been observed in this area of apparently "active exchange." These processes and their narrow extensions, the projections, are of special interest and will be discussed in detail.

The ultrastructural characteristics of these processes (microtubules, mitochondria, MVB, complex granules) are suggestive of nerve tissue. However, it is difficult to identify nerve tissue ". . . by means of a single electron micrograph or even a small collection of electron micrographs. . . . The cells (nerve cells) are all relatively large, many are multipolar and highly irregular in shape." (Peters et al., '70).

Some observations indicate that nervous tissue is found in the vicinity of intestine in barnacles (Cannon, '47; Cornwall, '53; Horridge, '65). Correlation of thick paraffin sections with thick and thin epon sections should provide additional information on the extent of the nervous system in these organisms.

Two distinct types of terminalization appear to occur between ends of the processes and basal surface of intestinal epithelium in the midgut region of barnacles. The first type of terminalization involves portions of processes (not projections) which lie between the basal lamina and the basal end of intestinal epithelium (fig. 11). The relationship between the structures in this type of terminalization is similar to that observed in parotid gland (Hand, '70), in bronchial glands (Bensch et al., '65) in human labial salivary glands (Tandler and Ross, '69), in lacrimal glands (Yamauchi and Burnstock, '67) in sensory epithelium in tongue (Farbman, '65) and is suggestive of "direct-contact" innervation (Grillo, '66).

However, in the present study, it is difficult to distinguish between what has been described as "segregated" area at the basal end of the epithelial cell and portions of the cell process. Both cytoplasmic regions ("segregated" area and process) contain similarly appearing heteromorphic vesicles and microtubules (figs. 4, 5, 6, 11). Indeed, that the "segregated" area may be expanded portions of the cell process within complex inpocketings of the intestinal epithelial cell surface is suggested by: (1) differences in alignment of microtubules in the "segregated" area compared with that in the surrounding epithelial cell, (2) the cytoplasmic background in both the "segregated" area and process is, generally, electron-lucent while that in the surrounding epithelial cell is more electron-dense, (3) the "segregated" area is confined by a double rather than single membrane.

The second type of terminalization, that involving narrow, finger-like extensions from the cell process, the *projections*, (which penetrate the basal lamina and end as dilated bulbs within inpocketings of the intestinal epithelium (figs. 14, 15), do not fulfill established morphological criteria for neural end-processes (Peters et al., '70). The projection is extremely narrow (ca. 500 Å) and its minute end-bulb does not contain any type of vesicle.

There is some similarity between the projection described in this study and "secondary myoepithelial cell processes lying within depressions of secretory cells" at the periphery of the acinus in rat submaxillary glands (fig. 8, Tamarin, '66). However, the

secondary myoepithelial cell processes contain fine filaments whereas processes that give rise to the projections contain microtubules.

Clusters of small vesicles, ca. 400 Å in diameter, are consistently observed at synapses between end-process and secretory epithelium (Tandler and Ross, '69; Yamauchi and Burnstock, '67). Occasionally larger heteromorphic vesicles, ca. 700 Å to 1,200 Å in diameter, are interspersed with the smaller vesicles (Bensch et al., '65; Tandler and Ross, '69). Cluster of the smaller 400 Å vesicles were not observed in the midgut region in barnacle material in the present study nor were they observed in end-processes of sensory epithelium in tongue (Farbman, '65). However, in the present study, larger heteromorphic granules were observed along the length of cell processes (abundant in *B. balanoides*), in portions of processes lying closely apposed to surface of intestinal epithelium (fig. 11) and in what has been tentatively termed "segregated areas" at the basal end of intestinal epithelium (figs. 4, 5, 6).

These heteromorphic vesicles appear similar in organization to elementary granules, the secretory granules of neurosecretory cells (Simpson, '66; Bargmann, '66) and to some granules containing neurotransmitter substances in sympathetic neurons (Grillo, '66; Richardson, '64). Similarly appearing granules may also belong to the lysosome system (Lane, '66). The latter are, in general, readily identified with appropriate cytochemical techniques for marker enzymes (Lane, '66). Neurosecretory granules are more difficult to characterize cytochemically (Simpson et al., '66).

The close structural relationships between the "neural-like" cell processes and the basal end of the intestinal epithelium in the balanidae appear to be unique. The significance of these relationships is not presently understood and must await additional studies on the nature of the cells that give rise to the processes as well as on the nature of the heteromorphic vesicles which they contain.

LITERATURE CITED

Anderson, E., and W. R. Harvey 1966 Active transport by the cecropia midgut. II. Fine structure of the midgut epithelium. J. Cell Biol., *31:* 107–134.

Bargmann, W. 1966 Neurosecretion. Int'l. Rev. Cytol., *19:* 183–201.

Barka, T., and P. J. Anderson 1965 Histochemistry: Theory, Practice and Bibliography. Hoeber, New York, pp. 65–95.

Bennett, D., and O. Radimska 1966 Flotation-fluid staining; toluidine blue applied to maraglas sections. Stain Technology, *41:* 349–350.

Bensch, K. G., G. B. Gordon and L. R. Miller 1965 Studies on the bronchial counterpart of the kultschitzky (Argentaffin) cell and innervation of bronchial glands. J. of Ultrastruct. Res., *12:* 668–686.

Cannon, H. G. 1947 On the anatomy of the pedunculate barnacle, *Lithothyra*. Phil. Trans. (B), *233:* 89–136.

Cornwall, I. E. 1953 The central nervous system of barnacles (Cirripedia). J. Fisheries Res. Board of Canada, *10:* 76–84.

Farbman, A. I. 1965 Fine structure of the taste bud. J. of Ultrastruct. Res., *12:* 328–350.

Grillo, M. A. 1966 Electron microscopy and sympathetic tissues. Pharmacol. Rev., *18:* 387–399.

Hand, A. R. 1970 Nerve-acinar cell relationships in the rat parotid gland. J.C.B., *47:* 540–543.

Horridge, G. A. 1965 Arthropoda: General Anatomy. In: Structure and Function in the Nervous System of Invertebrates. Vol. 2. T. H. Bullock and G. A. Horridge, eds. W. H. Freeman and Co., San Francisco, pp. 801–964.

Koulish, S. 1969 Observations on the origin of two substances produced by the vitellaria in *Gorgoderina attenuata*. J. Morph., *127:* 431–438.

Lane, N. J. 1966 The fine-structural localization of phosphatases in neurosecretory cells within the ganglia of certain gastropod snails. Amer. Zool., *6:* 139–157.

Luft, J. H. 1961 Improvement in epoxy resin embedding methods. J. Biophys. Biochem. Cytol., *9:* 409–414.

Newman, W. A., V. A. Zullo and T. H. Withers 1969 Cirripedia. In: Treatise on Invertebrate Paleontology. Part R, Arthropoda 4, Vol. 1. R. C. Moore, ed., Geol. Soc. of America, U. Kansas, pp. 206–295.

Pease, D. C. 1956 Infolded basal plasma membranes found in epithelia noted for their water transport. J. Biophys. and Biochem. Cytol., *2:* 203–208.

Peters, A., S. L. Palay and H. de F. Webster 1970 The Fine Structure of the Nervous System. The Cells and Their Processes. Hoeber, New York.

Richardson, K. C. 1964 The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscles. Am. J. Anat., *114:* 173–206.

Simpson, L., H. A. Bern and R. Nishioka 1966 Survey of evidence for neurosecretion in gastropod molluscs. Amer. Zool., 6: 123–138.

Tamarin, A. 1966 Myoepithelium of the rat submaxillary gland. J. Ultrastruct. Res., 16: 320–338.

Tandler, B., and L. L. Ross 1969 Observations of nerve terminals in human labial salivary glands. J.C.B., 42: 339–343.

Venable, J. H., and R. Coggeshall 1965 A simplified lead citrate stain for use in electron microscopy. J. Cell Biol., 25: 407–408.

Yamauchi, A., and G. Burnstock 1967 Nerve-myoepithelium and nerve-granular epithelium contacts in the lacrimal gland of the sheep. J.C.B., 34: 917–919.