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William E. Hawkins
Gulf Coast Research Laboratory, William.Hawkins@usm.edu

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ULTRASTRUCTURE OF RODLET CELLS: RESPONSE TO CADMIUM DAMAGE IN THE KIDNEY OF THE SPOT *LEIOSTOMUS XANTHURUS* LACÉPÈDE

WILLIAM E. HAWKINS
Microscopy Section, Gulf Coast Research Laboratory, Ocean Springs, Mississippi 39564

ABSTRACT Rodlet cell ultrastructure was studied in normal and cadmium-damaged kidney tissues of the spot *Leiostomus xanthurus*, an estuarine teleost. Rodlet cells in control fish occurred in all parts of the nephron except the renal corpuscle, were oblong to pear-shaped (about 5X10 μm), and contained up to 30 rodlet bodies, a basally situated nucleus, poorly developed mitochondria, and a filamentous cortex. Desmosomes and tight junctions joined rodlet cells to kidney epithelial cells. After cadmium exposure, rodlet cells showed a range of responses from secretory stimulation to necrosis. Rodlet bodies, which were membrane-bound, club-shaped granules, were secreted by a merocrine process, apparently aided by contraction of the filamentous cortex. New rodlet bodies were assembled in the Golgi apparatus. Mitochondria hypertrophied and developed well-defined cristae. The ultrastructural organization of the rodlet cells in this study and their responses to stimuli suggest that these are tissue or host cells rather than parasites as proposed by some authors. Further studies, however, are needed to confirm the nature of these cells.

INTRODUCTION

Rodlet cells occur frequently in fish tissues and have long been the subject of controversy over whether they are protozoan parasites (Thélohan 1892) or tissue cells (Plehn 1906). Most ultrastructural studies agree that the principal cytologic features of these cells are rodlet bodies, a filamentous cortex that lies beneath the plasma membrane, and a basally situated nucleus. However, there is little agreement on the nature of the rodlet cell and reports on ultrastructural details vary. Some consider the cells to be parasites because of their widespread distribution in tissues or because of the resemblance of some rodlet cell organelles to those of apicomplexan protozoans (Bannister 1966, Iwai 1968, Mourier 1970, Flood et al. 1975, Mayberry et al. 1979). Others consider rodlet cells to be unicellular glands in which the rodlet bodies are secretory granules formed in the Golgi apparatus from material synthesized in the rough endoplasmic reticulum (RER) (Leino 1974, Desser and Lester 1975, Morrison and Odense 1978, Mattey et al. 1979).

In most studies, rodlet cells have been examined in normal tissues. Few studies have involved rodlet cells in pathological or toxicological situations. In a study on the effect of cadmium on the kidney of the spot *Leiostomus xanthurus*, an estuarine teleost (Hawkins et al. 1980), we found that parts of the renal tubule had abundant rodlet cells. Since cadmium caused severe damage to renal tubular epithelial cells, we thought it worthwhile to examine the ultrastructural changes in rodlet cells in cadmium-damaged renal tubules.

MATERIALS AND METHODS

Twenty-six spot, 10–15 cm in total length, were collected by trawl, seine, and hook and line from the Mississippi Sound. Specimens were taken to the laboratory and either processed immediately or maintained in glass aquaria containing filtered and circulating artificial sea water with a salinity of 15–25 ppt. Fish were killed by pithing. Kidneys were fixed *in situ* in either 3.0% glutaraldehyde in 0.1 M phosphate buffer or in Karnovsky's fixative (Karnovsky 1965) in 0.1 M cacodylate buffer. For transmission electron microscopy (TEM), tissues were minced, rinsed in the appropriate buffer and postfixed in 1.0% osmium tetroxide. Some tissues were *en bloc* stained with aqueous uranyl acetate. Tissues were dehydrated in ethanol and embedded in epoxy resin. Thin sections were stained with lead citrate and examined with a Phillips 301 or Siemens Elmiskop 1A electron microscope. For orientation, semithin sections (1–2 μm thickness) were cut, mounted on glass slides, and stained with toluidine blue.

For scanning electron microscopy (SEM), whole kidneys were dissected as described above and allowed to fix for 2 h to several days and then cut with a razor blade into sections about 2 mm thick. The sections were postfixed for 1 h in buffered 1.0% osmium tetroxide, dehydrated in ethanol and critical point dried using CO_2. Tissues were sputter-coated with gold and examined with an ETEC Autoscan.

Procedures for exposing fish to cadmium have been described (Hawkins et al. 1980). Briefly, spot were exposed in static aquaria to levels of cadmium chloride from 1 to 100 ppm for 48 h. Tissues were processed for electron microscopy as described above.

RESULTS

Rodlet cells in control kidney

Rodlet cells occurred in the epithelium of the neck segment, proximal tubule, collecting tubule, and ureteric duct. In control kidneys, rodlet cells were not found in renal corpuscles, blood vessels, or hemopoietic tissues. SEM of

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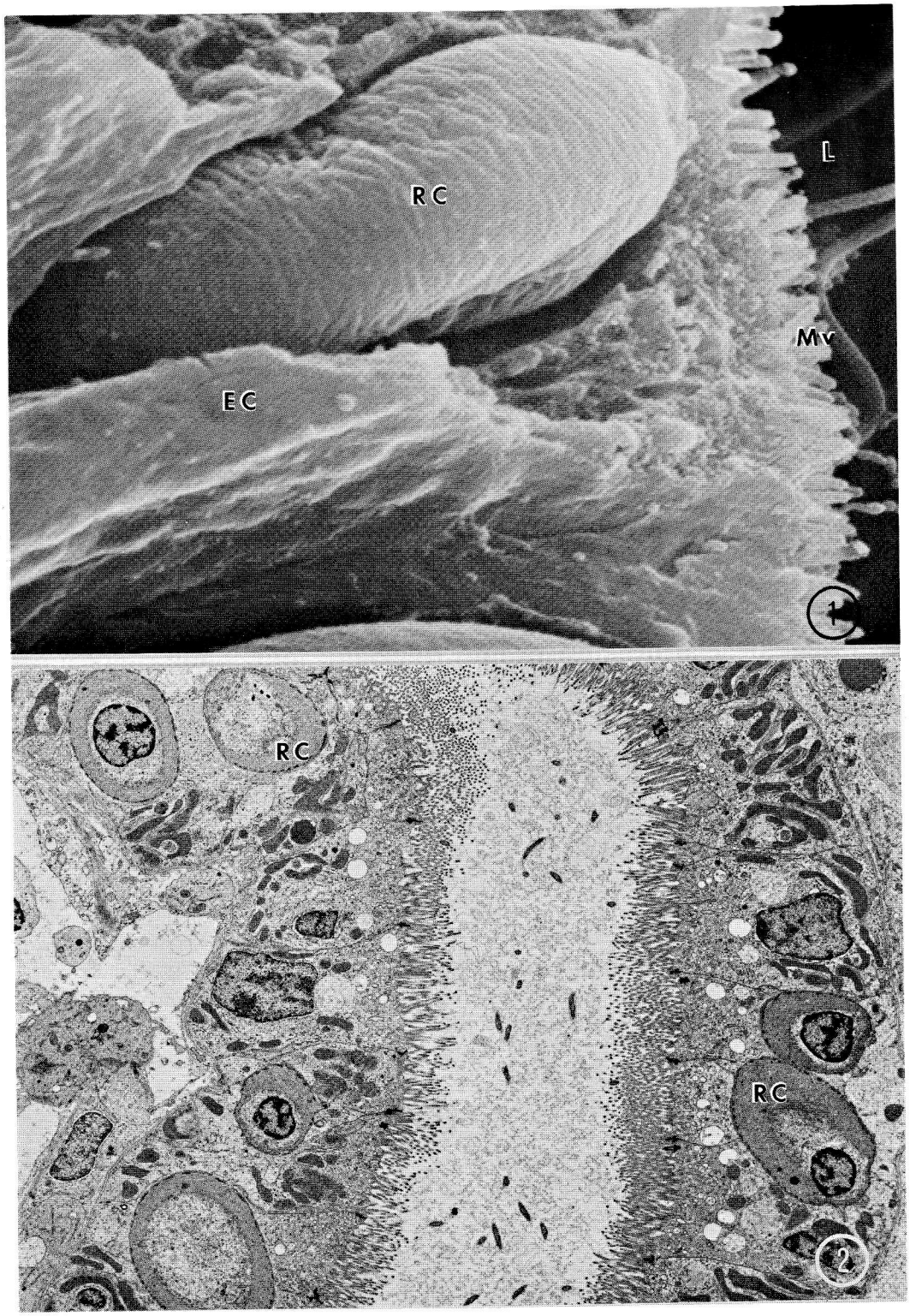

Figure 1. Scanning electron micrograph of a rodlet cell (RC) between epithelial cells (EC) of the ureteric duct. Note ridges and furrows on surface of rodlet cell. Duct lumen (L); microvilli (Mv). X 13,200
Figure 2. Transmission electron micrograph of rodlet cells (RC) in proximal tubule. X 3,200

ureteric duct epithelium showed that the rodlet cells were oblong to pear-shaped and wedged between the epithelial cells (Figure 1). The rodlet cell surface formed circumferential ridges and furrows. The apex of the rodlet cell often bordered on the lumen and occasionally issued microvillus-like processes into the duct lumen. The space that separated the rodlet cell from surrounding epithelial cells was not present in TEM samples or in SEM samples that were prepared by freeze-cracking (unpublished observations). In some proximal tubules, rodlet cells (about 5X10 μm) were almost as abundant as tubule epithelial cells (Figure 2).

The structure and organization of most rodlet cell organelles conformed with those of other species. Some important features are described for comparison with cadmium-exposed cells but are not illustrated.

The rodlet cell apex faced the tubule lumen. The basally situated nucleus (3–4 μm in diameter) contained dense, marginated chromatin. As many as 30 club-shaped membrane-bound rodlet bodies, each with an electron-dense core, extended from near the nucleus to the cell apex. Golgi complexes were rarely seen. Elongate, sinuous, poorly differentiated mitochondria (about 0.15 to 0.30 μm in diameter) occurred near the apex. A filamentous cortex (about 0.5 μm thick) which lay beneath the plasmalemma, except at the apex, contained thick filaments (20 nm in diameter) oriented around the long axis of the cell and thin filaments (6–8 nm in diameter) that were not regularly oriented. Microtubules (about 10 nm in diameter) ran along the inner aspect of the cortex from the apical to the basal region. Dense plaques situated 15–20 nm from the plasmalemma lined the cell at regular intervals. Rodlet cells and tubular epithelial cells were connected by desmosomes and tight junctions.

Some rodlet cells appeared open to the tubule lumen (Figure 3). Membrane-bound rodlet bodies were seen in the tubule lumen near such cells. The nuclei of these rodlet cells resembled those of normal resting cells. Usually, the tubule lumens that contained rodlet cell debris were compressed and also contained debris from the tubular epithelium.

Cadmium-exposed kidney

Exposure to cadmium levels greater than 10 ppm for 48 h damaged proximal tubular epithelium (Hawkins et al. 1980). Concurrently, changes took place in rodlet cells. Rodlet cells were not disrupted or damaged as severely as the tubular epithelial cells. Detached rodlet cells, however, lay among epithelial cells, in tubule lumens, and in Bowman's space of the renal corpuscle (Figure 4). Some rodlet cells were joined by desmosomes (Figure 5). In many cells, the filamentous cortex was thickened and the dense plaques were nearly continuous. Vesicles often occurred within the filamentous cortex or between it and the plasmalemma. Some rodlet cells appeared to expel their rodlet bodies by a merocrine process whereby the membrane surrounding a rodlet body became continuous with the plasmalemma (Figure 6). Other organelles of these secreting cells were similar to those of resting cells.

Rodlet cells appeared to reform rodlet bodies in the Golgi apparatus (Figure 7). The dense core of forming rodlet bodies was smaller than in mature rodlet bodies. The origin of the dense core was not determined. RER was abundant in the supranuclear cytoplasm, especially near developing rodlet bodies. The mitochondria of these cells were rounder, larger, and cristae better developed than in control rodlet cells (Figure 8). Nucleoli, usually absent in control rodlet cells, were sometimes present in these cells.

In some rodlet cells, the area between the filamentous cortex and the cytoplasm was not distinct and the cortex lacked subplasmalemmal dense plaques. These cells often contained dense spherical structures 0.5 to 1.0 μm in diameter (Figures 4, 9, 10). Rodlet bodies were similar to those in control rodlet cells. Mitochondria were often swollen and vacuolated. Membrane-bound inclusions of homogenous material, membranes, and vesicles frequently occurred in these cells. Centrioles were often present in the apical cytoplasm (Figure 10). Nuclei contained one or more dense spherical inclusions that were as large as 2.0 μm in diameter. Otherwise, the nucleus was electron lucent with a flocculent nucleoplasm (Figure 9).

Many rodlet cells appeared to be in late stages of necrosis. The plasmalemma was often disrupted, especially at the apex. Nuclei were pyknotic and sometimes in the process of being expelled (Figure 11). The fibrillar cortex was intact although dense plaques were lacking. Also lacking were the microtubules that ran in the junction between the cytoplasmic core and the fibrillar cortex. Mitochondria were round with prominent cristae and a few dense deposits. Occasionally, degenerating rodlet cells were phagocytosed by monocytic macrophages (Figure 12).

DISCUSSION

The origin and functions of rodlet cell mitochondria and rodlet bodies are disputed by the tissue-cell (Leino 1974, Desser and Lester 1975, Morrison and Odense 1978, Mattey et al. 1979) and parasite (Bannister 1966, Mourier 1970, Mayberry et al. 1979) proponents. Distinct, ovoid mitochondria with prominent cristae occur in immature, developing rodlet cells (Leino 1974, Desser and Lester 1975) whereas mitochondria in mature rodlet cells are tubular with indistinct cristae (Bannister 1966, Wilson and Westerman 1967, Mourier 1970, Leino 1974, Desser and Lester 1975, Morrison and Odense 1978, Barber et al. 1979). Mayberry et al. (1979) described structures reported to be mitochondria in mature rodlet cells as micronemes. Micronemes are osmiophilic, cord-like organelles of apicomplexan parasites (Chobotar and Scholtyseck 1982). Rodlet cells in control spot kidney had tubular mitochondria with indistinct cristae similar to the mitochondria in mature rodlet cells of other species. After exposure to nephrotoxic

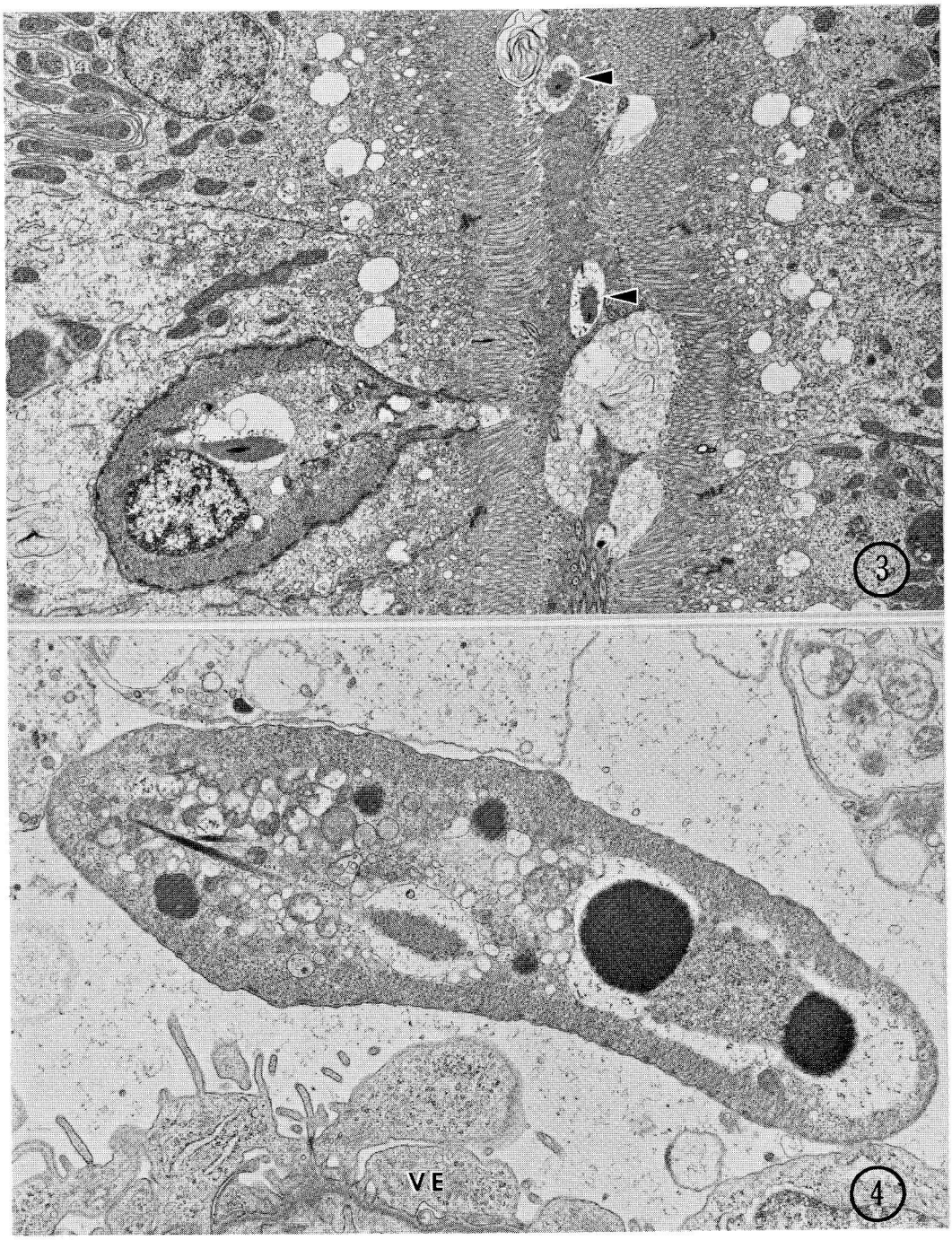

Figure 3. Membrane-bound rodlet bodies (arrowheads) in lumen of proximal tubule. Note apex of rodlet cell appears to open into lumen. Also note other debris in lumen. X 4,100

Figure 4. Detached rodlet cell in Bowman's space following cadmium exposure. Note dense nuclear and cytoplasmic bodies in rodlet cell. Visceral glomerular epithelium (VE). X 6,400

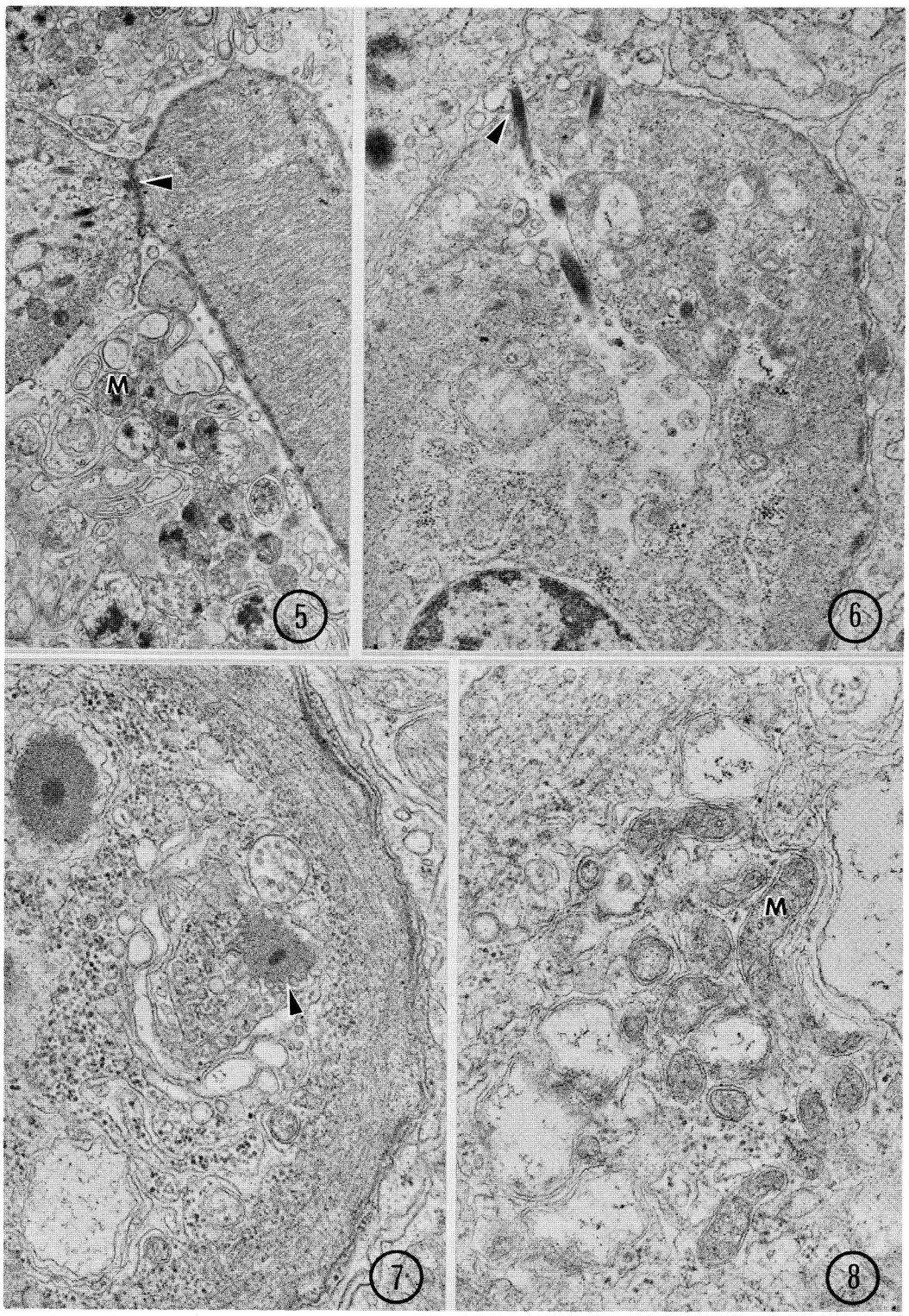

Figure 5. Desmosome (arrowhead) between two rodlet cells. Note disrupted and damaged mitochondria (M) of renal tubule epithelium. Cadmium-exposed. X 8,400

Figure 6. Rodlet body apparently being secreted without disruption of plasma membrane. Note that at the arrowhead, the plasma membrane becomes continuous with membranes of the rodlet body vacuole. Cadmium-exposed. X 17,300

Figure 7. Immature rodlet body (arrowhead) associated with Golgi-like membranes and vesicles. Cadmium-exposed. X 27,600

Figure 8. Rodlet cell mitochondria (M) following cadmium damage. X 30,800

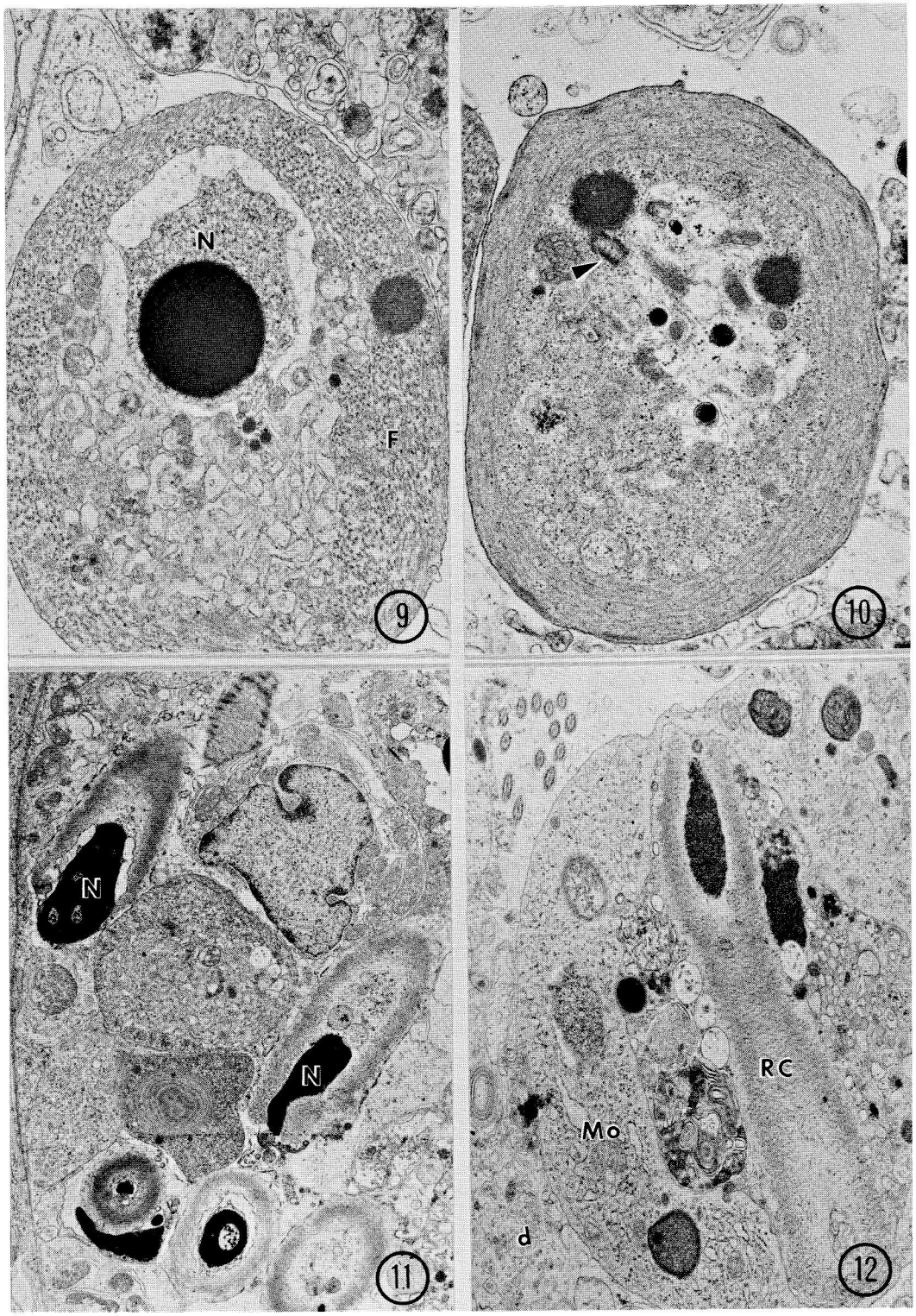

Figure 9. Rodlet cell following cadmium damage showing swollen filamentous cortex (F) and dense bodies in nucleus (N) and cytoplasm. X 12,800

Figure 10. Centriole (arrowhead) in rodlet cell following cadmium exposure. X 15,000

Figure 11. Necrotic rodlet cells following cadmium exposure. Note pyknotic nuclei (N). X 4,000

Figure 12. Rodlet cell (RC) phagocytosed by monocyte (Mo) following cadmium exposure. X 6,600

cadmium levels, however, the tubular mitochondria became ovoid with prominent cristae. Thus, these structures were clearly identifiable as mitochondria.

Cytochemical studies of nucleic acids in rodlet bodies disagree. Leino (1982) identified carbohydrates and protein in the granular matrix of the rodlet body and determined that the rodlet core contained protein but no carbohydrate or nucleic acids. Based on RNAase digestion studies, Barber et al. (1979) suggested that rodlet body cores contained RNA. Bielek and Viehberger (1983), supporting the parasitic nature of rodlet cells, identified DNA in rodlet cores by fluorescence staining and DNAase digestion studies.

Several studies showed that rodlet bodies are synthesized in Golgi apparatus of immature cells (Leino 1974, Desser and Lester 1975, Barber et al. 1979, Mattey et al. 1979). In spot exposed to cadmium, rodlet cells apparently were stimulated to secrete their rodlet bodies which were replaced by the Golgi apparatus. The release of rodlet bodies occurred by a merocrine process without disruption of the plasmalemma and was accompanied by contraction of the filamentous cortex. Leino (1974) suggested that rodlet cell secretion was holocrine and that the secretion involved contraction of the filamentous cortex, disruption of the apical plasmalemma, and expulsion of the rodlet cell contents. Mayberry et al. (1979) who referred to rodlet bodies as rhoptries, coccidian organelles that appear to aid in the penetration of the host cell by the coccidium (Chobotar and Scholtyseck 1982), observed rodlet cell organelles and whole rodlet cells in the lumens of epithelial tissues and suggested that this resulted from handling or processing damage whereas intact rodlet cells were parasites that had left the host tissues. Mattey et al. (1979) also maintained that the appearance of holocrine secretion by rodlet cells was the result of handling or fixation damage. In the spot, holocrine secretion by rodlet cells also appears to be artifactual because tubule lumens that contained rodlet cell debris often contained epithelial cell debris as well. However, it is possible, as Leino (1974) suggested, that sloughing of most or all of the rodlet cell contents is the final stage of the cycle of this cell. If the normal secretion of the rodlet cell is merocrine, then the function of the filamentous cortex is not clear. Perhaps contraction of the filamentous cortex is necessary to aid in expelling the large rodlet body with its apparently rigid core.

Rodlet cell junctional complexes vary among species. Desmosomes occur between rodlet cells and epithelial cells in several species of freshwater fishes (Leino 1974, Mattey et al. 1979) and tight junctions between rodlet cells and epithelial cells in the operculum and gill raker of the white sucker *Catostomus commersoni* Lacépède (Desser and Lester 1975). Mourier (1970), who considered rodlet cells to be parasites, reported desmosomes between rodlet cells but not between rodlet cells and tubule epithelial cells in the kidney of the stickleback *Gasterosteus aculeatus* L. Rodlet cells in cadmium-damaged spot kidney were occasionally joined by desmosomes although such junctions in normal kidney were not observed. The significance of this is not clear. Intercellular junctions were not reported between immature or developing rodlet cells or between such cells and epithelial cells by Leino (1974) or Desser and Lester (1975). The ability of rodlet cells to form desmosomes and tight junctions is not shared with any apicomplexan parasite.

The present study confirms neither the parasitic nor the tissue-cell nature of the rodlet cell. Confirmation must await studies characterizing rodlet cell DNA and immunological properties and comparing these with known fish cells. It is likely that preparations rich in rodlet cells such as the spot proximal tubule could be exploited for these studies. Nevertheless, the ultrastructural organization of rodlet cells in control and cadmium-damaged renal tubules of the spot suggests to us that these are tissue cells rather than apicomplexan parasites.

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REFERENCES CITED

Bannister, L. H. 1966. Is *Rhabdospora thelohani* (Laguesse) a sporozoan parasite or a tissue cell of lower vertebrates? *Parasitology* 56:633–638.

Barber, D. L., J. E. M. Westermann & D. N. Jensen. 1979. New observations on the rodlet cell (*Rhabdospora thelohani*) in the white sucker *Catostomus commersoni* (Lacépède): LM and EM studies. *J. Fish Biol.* 14:277–284.

Bielek, E. & G. Viehberger. 1983. New aspects on the "rodlet cell" in teleosts. *J. Submicrosc. Cytol.* 15:681–694.

Chobotar, B. & E. Scholtyseck. 1982. Ultrastructure. Pages 101–166 *in:* Long, P. L. (ed.), *The Biology of the Coccidia*. University Park Press, Baltimore, Maryland.

Desser, S. S. & R. Lester. 1975. An ultrastructural study of the enigmatic "rodlet cells" in the white sucker, *Catostomus commersoni* (Lacépède) (Pisces: Catostomidae). *Can. J. Zool.* 53:1483–1494.

Flood, M. T., R. F. Nigrelli & J. F. Gennaro. 1975. Some aspects of the ultrastructure of the "Stäbchendrüsenzellen," a peculiar cell associated with the endothelium of the bulbus arteriosus and with other fish tissues. *J. Fish Biol.* 7:129–138.

Hawkins, W. E., L. G. Tate & T. G. Sarphie. 1980. Acute effects of cadmium on the spot *Leiostomus xanthurus* (Teleostei): Tissue distribution and renal ultrastructure. *J. Toxicol. Environ. Health* 6:283–295.

Iwai, T. 1968. Notes on the pear-shaped cell (rodlet cell) in the epithelium of the digestive tract of fishes. *Bull. Jpn. Soc. Sci. Fish.* 34:133–137 (In Japanese).

Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* 27A:127–138.

Leino, R. L. 1974. Ultrastructure of immature, developing, and secretory rodlet cells in fish. *Cell Tissue Res.* 155:367–381.

Leino, R. L. 1982. Rodlet cells in the gill and intestine of *Catostomus commersoni* and *Perca flavescens:* A comparison of their light and electron microscopic cytochemistry with that of mucous and granular cells. *Can. J. Zool.* 60:2768–2782.

Mattey, D. L., M. Morgan & D. E. Wright. 1979. Distribution and development of rodlet cells in the gills and pseudobranch of the bass, *Dicentrachus labrax* (L). *J. Fish Biol.* 15:363–370.

Mayberry, L. F., A. A. Marchiondo, J. E. Ubelaker & D. Kazic. 1979. *Rhabdospora thelohani* Laguessé, 1895 (Apicomplexa): New host and geographic records with taxonomic considerations. *J. Protozool.* 26:168–178.

Morrison, C. M. & P. H. Odense. 1978. Distribution and morphology of the rodlet cell in fish. *J. Fish. Res. Board Can.* 35:101–116.

Mourier, J. -P. 1970. Structure fine de *Rhabdospora thelohani* Henneguy, protiste parasite de *Gasterosteus aculeatus* L. *Z. Parasitenkd.* 34:198–206.

Plehn, M. 1906. Ueber eigentumliche drüsenzellen im gefässsystem und in anderen organen bei fischen. *Anat. Anz.* 28:192–203.

Thélohan, P. 1892. Sur des sporozoaires indéterminés, parasites des poissons. *J. Anat. Phys. (Paris)* 38:163–171.

Wilson, J. A. F. & R. A. Westerman. 1967. The fine structure of the olfactory mucosa and nerve in the teleost *Carassius carassius* L. *Z. Zellforsch. Mikrosk. Anat.* 83:196–206.