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THE CONNECTIVE TISSUE OF THE LOCUST RECTUM: AN ULTRASTRUCTURAL STUDY

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Abstract: This study was undertaken to provide ultrastructural descriptions of the connective tissue secreting cells that exist near the rectal pads in *Schistocerca gregaria.* The connective tissue space surrounding the rectal pads contains collagen fibers and three cell types: fibroblasts, strand-bearing connective tissue cells, and granular cells. The fibroblasts which produce collagen fibers and matrix contain extensive rough endoplasmic reticulum (RER), free ribosomes, a prominent Golgi complex, vesicles, and mitochondria. In actively secreting fibroblasts, the cisternae of the RER become dilated with amorphous electron-dense material. Smooth vesicles containing the electron-dense material approach and fuse with the plasma membrane, releasing their contents by exocytosis near the cell surface for assembly into collagen fibers and other matrix constituents. The strand-bearing connective tissue cells exhibit intracellular channels, abundant glycogen granules, and lipid droplets. Their fibrous strands extend into the connective tissue space. The reticular lamina surrounding the actively secreting fibroblasts and strand-bearing cells contains small, unbanded and banded, collagen fibers, which become dispersed in the connective tissue space. The granular cells resemble vertebrate mast cells, which contain heparin and histamine in their granules. The granular cells exhibit short pseudopodia, intracellular channels, and granules with varying densities. The ultrastructural features of the fibroblasts and strand-bearing connective tissue cells are consistent with their role in the synthesis of collagen fibers and matrix constituents, whereas the granular cells appear to be involved in local immunological reactions.

KEYWORDS: Collagen fibers, endoplasmic reticulum, fibroblasts, Golgi complex, granular cells, intracellular channels, lipid droplets, mitochondria, strands.

INTRODUCTION

In multicellular organisms, the internal tissues and organs are supported and held together by connective tissue, which consists of cells and fibers that are embedded in an amorphous matrix. The connective tissue functions in water storage, allows the passage of food substances and waste products, and forms layers of varying thickness, which allow movements between different tissues (Andrew, 1959; Leonhardt, 1977). One of most important and abundant classes of glycoprotein molecules produced by vertebrate fibroblasts is collagen. Collagen usually exists in the form of long fibers with well-defined banding patterns, and it acts as the chief packing material inside organs (Goldberg and Robinovitch, 1988).

In insects, hemocytes have been implicated in the formation of connective tissue. Wigglesworth (1956, 1973) demonstrated in *Rhodnius* (Hemiptera: Reduviidae) that amoebocytes are important in the formation of basement membranes. Scharrer (1972) reported that in cockroaches, hemocytes are involved in the formation of connective tissue. Two types of hemocytes (i.e., the adipohemocytes and spherule cells) are involved in the secretion of connective tissue fibers and strands associated with the tunica propria around the prothoracic gland in *Antheraea* (Lepidoptera: Attacidae) and *Bombyx* (Lepidoptera: Bombycidae) (Beaulaton, 1968). In the larva of *Sarcophaga* (Diptera: Sarcophagidae), Whitten (1964) found strands of connective tissue, which were probably secreted by hemocytes, connecting the pericardial cells to the heart. Insect ganglia are surrounded by an extracellular connective tissue sheath, the neural lamella, which is secreted by the underlying perineurium and contains collagen fibers in a matrix of acidic or neutral mucopolysaccharides and glycoproteins (Ashhurst, 1965, 1968, 1985; Locke and Huie, 1972; Smith and Treherne, 1963; Smith, 1968). The existence of connective tissue-secreting fibroblasts in a variety of insect tissues and organs is now well documented (Ashhurst and Costin, 1974; Francois, 1977, 1978; Hoffmann, *et al.,* 1979).

A stratum of loose connective tissue lies between the epithelium and the muscle layer in the rectal pads of *Calliphora erythrocephala* (Diptera: Calliphoridae) (Gupta and Berridge, 1966), *Periplaneta americana* (Dictyoptera: Blattidae) (Oschman and Wall, 1969), and *Locusta migratoria* (Orthoptera: Acrididae) (Hodge, 1939). In the rectal pads of *Schistocerca gregaria* (Orthoptera: Acrididae), connective tissue cells and bundles of collagen have been observed in the space between the secondary cells, which lie external to the epithelium, and the muscles (Jarial, 1992). Very little information is available about either the ultrastructure of these connective tissue cells or the synthesis of collagen fibers in the insect rectum. The fine structure of connective tissue cells surrounding the rectal pads in the desert locust, *Schistocerca gregaria,* and their relationship to the production of collagen fibers, the two matrix constituents (the glycosaminoglycans and glycoproteins), and other substances were investigated in this study.

MATERIALS AND METHODS

The adult male desert locusts (*Schistocerca gregaria*) used in this study were maintained at 28° C and a humidity of 60% on a diet of bran and lettuce leaves. For electron microscopy, the rectal pads were dissected out in insect Ringer solution (Hoyle, 1953) and fixed by immersion at room temperature in the following mixture (Millonig, 1962): 1 part 5% osmium tetroxide, 1 part 10% glutaraldehyde, and 2 parts 0.2M phosphate buffer (pH 7.2). After fixation, the material was washed in two changes of phosphate buffer, dehydrated in an ethanol series to propylene oxide, and embedded in Epon 812 (Luft, 1961). Ultra-thin sections were cut with a Porter-Blum MT2 ultramicrotome, stained with uranyl acetate, and counter-stained with lead citrate (Reynolds, 1963). The sections were examined using a Hitachi HU-11A and an H-600 transmission electron microscope. Similarly embedded material was also cut at 2 µm, stained with azure II, and viewed with a light microscope.

RESULTS

Each of the six rectal pads of *Schistocerca gregaria* is surrounded by an extensive (about 70 µm wide) connective tissue space, which is bounded by the layer of secondary cells which lies external to the epithelium and the visceral

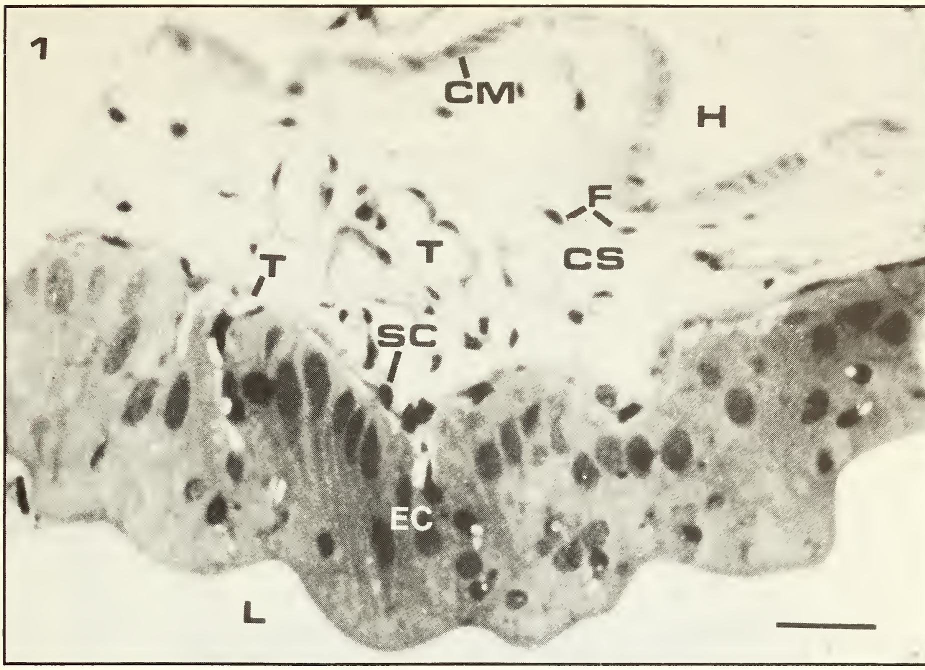
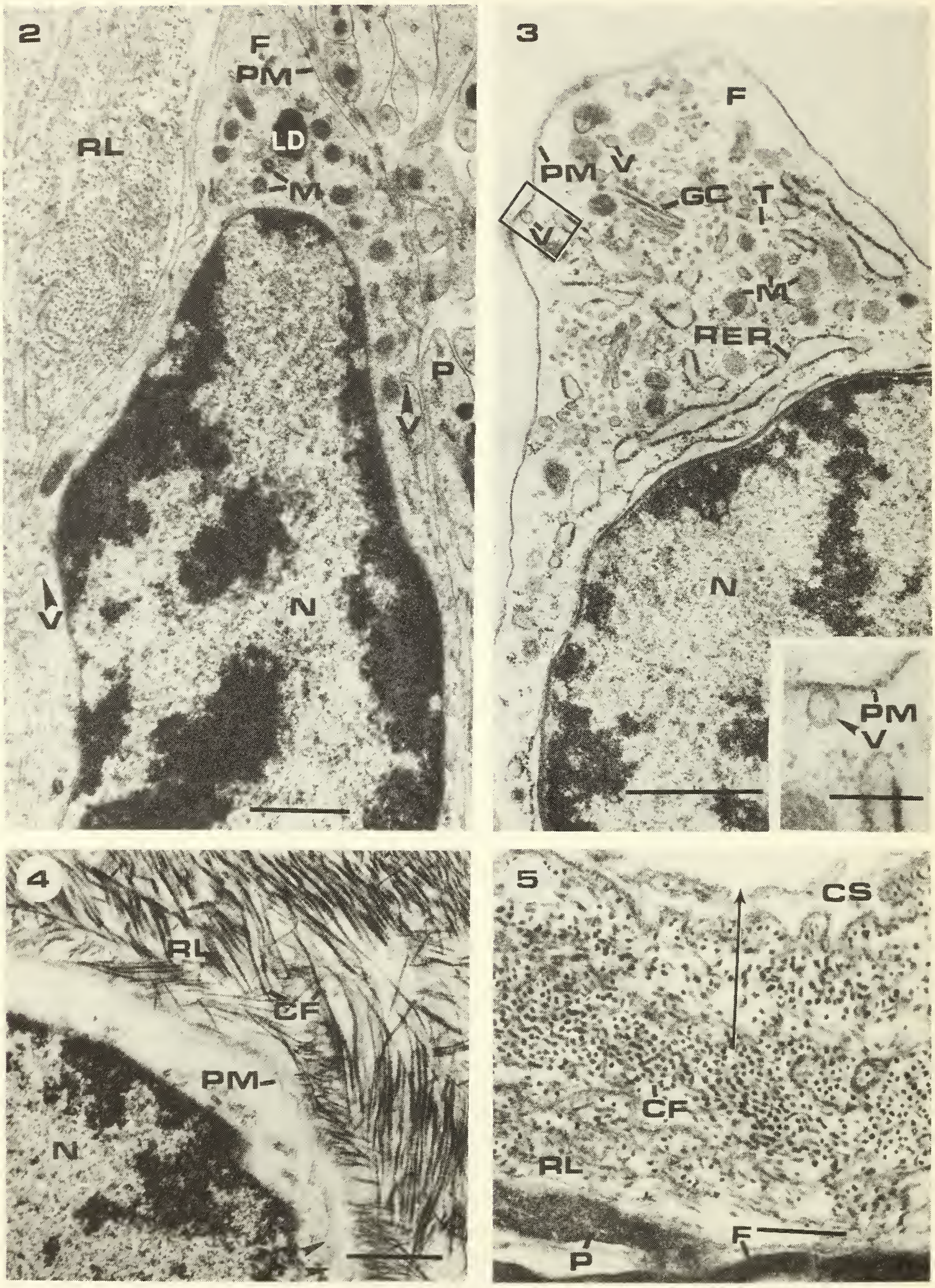


Figure 1. Light microscope photograph of a 2 μm thick Epon section of a rectal pad stained with azure II showing the extensive connective tissue space (CS) bounded by circular muscles (CM) and the layer of secondary cells (SC) and containing fibroblasts (F) and tracheae (T). EC = epithelial cells; H = hemocoel; L = lumen. Bar = 0.02 mm.

muscles. This space contains tracheae, tracheoles, connective tissue cells, and collagen fibers embedded in a matrix (Figures 1-5). Three cell types were recognized in the connective tissue space: fibroblasts, strand-bearing connective tissue cells, and granular cells.

The more numerous fibroblasts are spindle-shaped with slender cytoplasmic processes that extend into the surrounding connective tissue space. The ovoid nucleus of the fibroblast, which occupies a large portion of the cell, contains clumps of condensed chromatin adjacent to the nuclear membrane and scattered in the nucleoplasm (Figures 2-4). The thin layer of cytoplasm surrounding the nucleus contains extensive rough endoplasmic reticulum (RER), a prominent Golgi complex, vesicles, tubules, lipid droplets, and mitochondria. In actively secreting fibroblasts, the cisternae of the RER are dilated and filled with amorphous electron-dense material. Smooth vesicles, probably containing matrix constituents and procollagen, were observed approaching and then fusing with the plasma membrane thereby discharging their contents by exocytosis near the cell surface (Figures 2, 3). Small, unbanded and banded, densely packed collagen fibers (each about 30 nm in diameter) form in a reticular lamina (about 2 μm thick) surrounding the fibroblasts, whence they disperse into the connective tissue space (Figures 2, 4, 5).

The strand-bearing connective tissue cells display some structural features which differ from those of the fibroblasts described above. The plasma



Figures 2-5. Figure 2. A portion of a fibroblast (F) surrounded by a reticular lamina (RL) containing collagen fibers. Vesicles (V; arrow heads) are seen fusing with the plasma membrane (PM). LD = lipid droplet; M = mitochondria; N = nucleus; P = process of a fibroblast. Bar = 1 µm. **Figure 3.** Electron micrograph of a portion of fibroblast (F) exhibiting dilated rough endoplasmic reticulum (RER) filled with amorphous electron-dense material and a well-developed Golgi complex (GC). Note the vesicle (V) fusing with the plasma membrane (PM) in the area bounded by the

membrane of these cells is infolded to form narrow intracellular channels which extend deep into the cytoplasm and contain electron-dense material. The nucleus is centrally located, and the dense cytoplasm is devoid of granules but contains free ribosomes, vesicles, and numerous mitochondria. The cytoplasm is filled with glycogen granules and lipid droplets to such an extent that the organelles, especially the rough endoplasmic reticulum and the Golgi complex, become compressed and obscured from view. Smooth vesicles containing electron-dense material are visible near the intracellular channels and the plasma membrane. The slender cytoplasmic processes of these cells also extend into the connective tissue space. These connective tissue cells, like the active fibroblasts, are surrounded by a 750 nm thick reticular lamina containing thin collagen fibers. In addition, fibrous strands extend from the reticular lamina into the connective tissue space (Figure 6).

The granular cells are irregularly ovoid in section. These cells contain numerous, membrane-bound, round to oval, dense granules, a few profiles of rough endoplasmic reticulum, mitochondria, ribosomes, and vesicles in the cytoplasm surrounding the nucleus. The delicate plasma membrane is infolded to form intracellular channels. Short pseudopodia extend from the cell surface of granular cells, which gives an indication of their slow mobility (Figures 7, 8). In some preparations, the peripheral granules appear swollen and exhibit decreasing density (Figure 9).

DISCUSSION

Our current knowledge of the connective tissue in the internal organs of insects is rather scanty, since it has been assumed that the function of providing support has been largely taken over by the widely distributed tracheal system (Waterhouse, 1950). The rectal pads of the desert locust, *Schistocerca gregaria,* are surrounded by an extensive connective tissue space, which extends between the layer of secondary cells lying external to the epithelium and the visceral muscles. This space appears to be analogous to the lamina propria of the vertebrate gastrointestinal tract and contains connective tissue cells, collagen fibers, nerves, and trachea (Jarial, 1992). The connective tissue space contained three types of connective tissue cells: fibroblasts, strand-bearing connective tissue cells, and granular cells. The fibroblasts in the rectal pads of *Schistocerca* display ultrastructural features that are typical of vertebrate fibroblasts, suggesting that, like the latter, they are involved in the synthesis and deposition of collagen fibers

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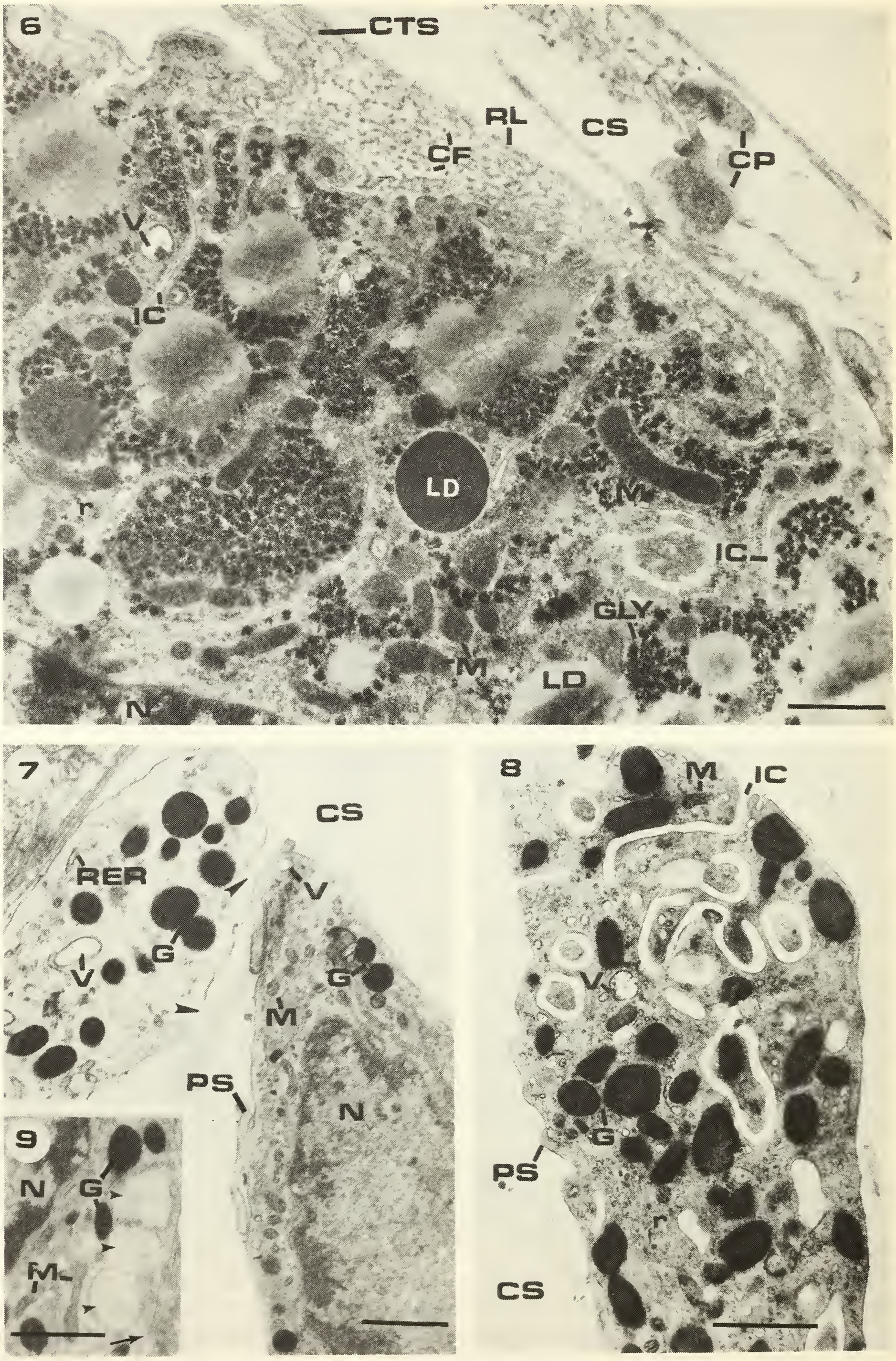
rectangle (M = mitochondria; N = nucleus; T = tubule; V = vesicles; Bar = 1 μm). The area corresponding to the rectangle is enlarged in the inset (V = vesicle containing electron-dense material; PM = plasma membrane; Bar = 0.25 μm). **Figure 4.** A portion of a fibroblast surrounded by a reticular lamina (RL) containing banded collagen fibers (CF) that are cut longitudinally. The plasma membrane (PM) has discontinuities (arrow heads at bottom). N = nucleus. Bar = 1 μm. **Figure 5.** Collagen fibers (CF) in the reticular lamina (RL) surrounding a fibroblast (F) and a process (P) of a different fibroblast. Almost all the fibers have been cut in cross section and appear as small dots. The direction of the arrow indicates dispersion of fibers into the connective tissue space (CS). Bar = 0.5 μm.



Figures 6-9. Figure 6. A portion of a strand-bearing connective tissue cell showing the intracellular channels (IC), glycogen deposits (GLY), lipid droplets (LD), mitochondria (M), vesicles (V), reticular lamina (RL), and connective tissue strands

and matrix constituents, namely the glycosaminoglycans and mucoproteins (Ashhurst, 1985). Similarly placed medullary cells in the rectal papillae of *Calliphora* have been shown to produce banded collagen fibers (Gupta in Ashhurst, 1968). In actively secreting fibroblasts, the cisternae of the rough endoplasmic reticulum (RER) are dilated and filled with electron-dense, amorphous material; the Golgi complex is prominent with well-developed lamellae; and the vesicles, presumably derived from the Golgi complex and containing electron-dense material, are situated near the RER. In these fibroblasts, the Golgi complex may play an important role in the synthesis of collagen fibers and the matrix. The smooth vesicles containing electron-dense material are transported to the periphery of the fibroblasts, where they fuse with the plasma membrane and discharge their contents to the exterior of the cells by exocytosis for assembly into collagen fibers and as matrix constituents. The thin, banded fibers (30 nm in diameter), which resemble type III collagen of vertebrates, are found in the reticular lamina surrounding the fibroblasts, but eventually the banded fibers are dispersed in the connective tissue space by mechanical forces.

The mechanism of collagen synthesis and elaboration of matrix constituents in insect fibroblasts appears to be identical to that described in vertebrate fibroblasts, where procollagen molecules synthesized in the RER cisternae are passed on to the Golgi complex for packaging, subsequently transported by vesicles to the cell surface, and then released to the exterior by exocytosis for further modification and assembly into collagen fibers (Bloom and Fawcett, 1986; Goldberg and Robinovitch, 1988). This view is supported by electron autoradiographic studies, which show that ^{3}H-proline taken up by insect fibroblasts appears in the RER and Golgi complex and is later incorporated into collagen fibers (Ashhurst and Costin, 1976; Francois, 1980).

The strand-bearing connective tissue cells in the rectal pads of *Schistocerca* structurally resemble the perineurial sheath cells, which surround the insect central nervous system, are responsible for the production of the collagen-rich neural lamella, and provide nourishment to the ganglia (Ashhurst, 1965; Smith, 1968; Smith and Treherne, 1963; Wigglesworth, 1960). The existence of intracellular channels in the strand-bearing cells suggests that they may also be involved in transporting substances like trehalose into these cells for synthesis of

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(CTS) containing collagen fibers (CF). CP = Connective tissue cell processes; CS = connective tissue space; N = nucleus; r = ribosomes. Bar = 1 μm. **Figure 7.** Portions of two granular cells in the connective tissue space (CS) exhibiting electron-dense granules (G), short pseudopodia (PS), a few profiles of rough endoplasmic reticulum (RER), mitochondria (M), and vesicles (V). The granular cell on the left has discontinuities in its plasma membrane (arrow heads). N = nucleus. Bar = 1 μm. **Figure 8.** Electron micrograph of a portion of a granular cell in the connective tissue space (CS) showing dense granules (G), intracellular channels (IC), mitochondria (M), vesicles (V), and blunt pseudopodia (PS). r = ribosomes. Bar = 1 μm. **Figure 9.** A small portion of a granular cell exhibiting electron-dense granules (G) and three granules (arrow heads) showing decreasing density located near the plasma membrane (arrow). M = mitochondria; N = nucleus. Bar = 1 μm.

the glycogen granules and lipid droplets, which completely fill the cytoplasm and compress organelles such as the RER. Even though the RER and the Golgi complex are obscured from view, the mechanism of collagen synthesis in these cells is probably similar to that in the fibroblasts. Electron-dense material seen in the cytoplasmic vesicles and in the adjoining intracellular channels may represent procollagen and/or matrix constituents about to be, or already, released by exocytosis. The connective tissue strands containing small collagen fibers, which extend from the reticular lamina of the connective tissue cells, most likely become attached to the nerves, tracheae, and tracheoles and anchor these structures in the connective tissue space. The glycogen deposits and lipid droplets of these cells may be utilized in providing nutrition to the underlying layer of secondary cells that have been implicated in active water uptake through the rectal pads (Jarial, 1992).

The granular cells in the rectal pads of *Schistocerca* possess ultrastructural features that appear similar to those of insect granulocytes, which play a role in encapsulation of foreign material and transplanted tissue (Han and Gupta, 1989), as well as to those of vertebrate mast cells (Goldberg and Robinovitch, 1988). The chemical nature of their dense granules is not known. The decreasing density displayed by peripheral granules in some preparations suggests that they undergo degranulation like those in vertebrate mast cells. The granular cells may produce substances which initiate or modify local immunological reactions.

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