CELL JUNCTIONS IN OMMATIDIA OF *LIMULUS*

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ABSTRACT

The intercellular relationships in the ommatidia of the lateral eye of *Limulus* have been investigated. The distal process of the eccentric cell gives origin to microvilli which interdigitate with the microvilli of the retinular cells. Therefore, both types of visual cells contribute to form the rhabdom and may have an analogous photoreceptor function. Quintuple-layered junctions are found within the rhabdom at the lines of demarcation between adjoining microvilli, whether the microvilli originate from a single retinular cell, from two adjacent retinular cells, or from a retinular cell and the eccentric cell. Furthermore, quintuple-layered junctions between the eccentric cell and the tips of the microvilli of the retinular cells occur at the boundary between the distal process and the rhabdom. These findings are interpreted to indicate that the rhabdom provides an extensive electrotonic junction relating retinular cells to one another and to the eccentric cell. Quintuple-layered junctions between glial and visual cells, as well as other structural features of the ommatidial cells, are also described.

INTRODUCTION

The fine structure of the ommatidia of the lateral eye of *Limulus polyphemus* has been already reported by Miller (20) who described the organization of the rhabdom and the nature of its component units. These observations, together with earlier light microscopic work (3, 13, 37, 38), are the morphological counterpart to the extensive physiological studies performed on the eye of *Limulus* (10, 13, 35, 38), and provide clues to some of the problems encountered in interpreting the electrical recordings. However, recent findings on the functional relationship between *Limulus* visual cells have indicated the need for further morphological investigation.

According to Smith et al. (34) retinular cells are electrically connected to one another and to the eccentric cell. When electrotonic transmission occurs in a variety of tissues, the existence of specialized junctional areas between cells can usually be demonstrated by electron microscopy (1, 4, 16, 31, 40). The present observations were undertaken, therefore, to investigate the possible existence of specialized junctions between visual cells in ommatidia of *Limulus*. This expectation was confirmed and, furthermore, similar intercellular junctions were detected between glial and visual cells. Some hitherto unknown structural features of the visual cells were also observed.

METHODS

Thin slices of the lateral eye of *Limulus polyphemus* were fixed for 6–16 hr in cold 3% glutaraldehyde (33) in 0.1 M phosphate buffer (pH 7.5) with 0.4 M sucrose. The tissue was then washed in the phosphate buffer with 0.6 M sucrose and postfixed for 2 hr in cold 1% OsO_4 in the phosphate buffer with or without sucrose. The glutaraldehyde solution was osmotically equivalent to sea water (25). Two hypertonic glutaraldehyde fixatives were also used. One of them contained 0.25 M NaCl in addition to the above components. Only Figs. 1 and 2 represent tissue fixed under such

conditions. To make up the other hypertonic fixative 0.6 M sucrose was added to the glutaraldehyde and phosphate buffer. Micrographs of tissue fixed in this medium are not presented here. The cell junctions to be described remained unchanged, however, after fixation in either hypertonic mixture.

After fixation, some blocks of tissue were stained in an aqueous uranyl acetate solution (8, 17). All the specimens were dehydrated in graded ethanol and embedded in Epon (19). The sections were all doubly stained with uranyl acetate (39) and lead citrate (27). Since the only variation in the processing was the staining in block with uranyl, the use of this method is noted when pertinent. Observations were made with an RCA EMU 3G microscope.

OBSERVATIONS

Information on the microscopic anatomy of the ommatidia is already available (20, 37, 38).

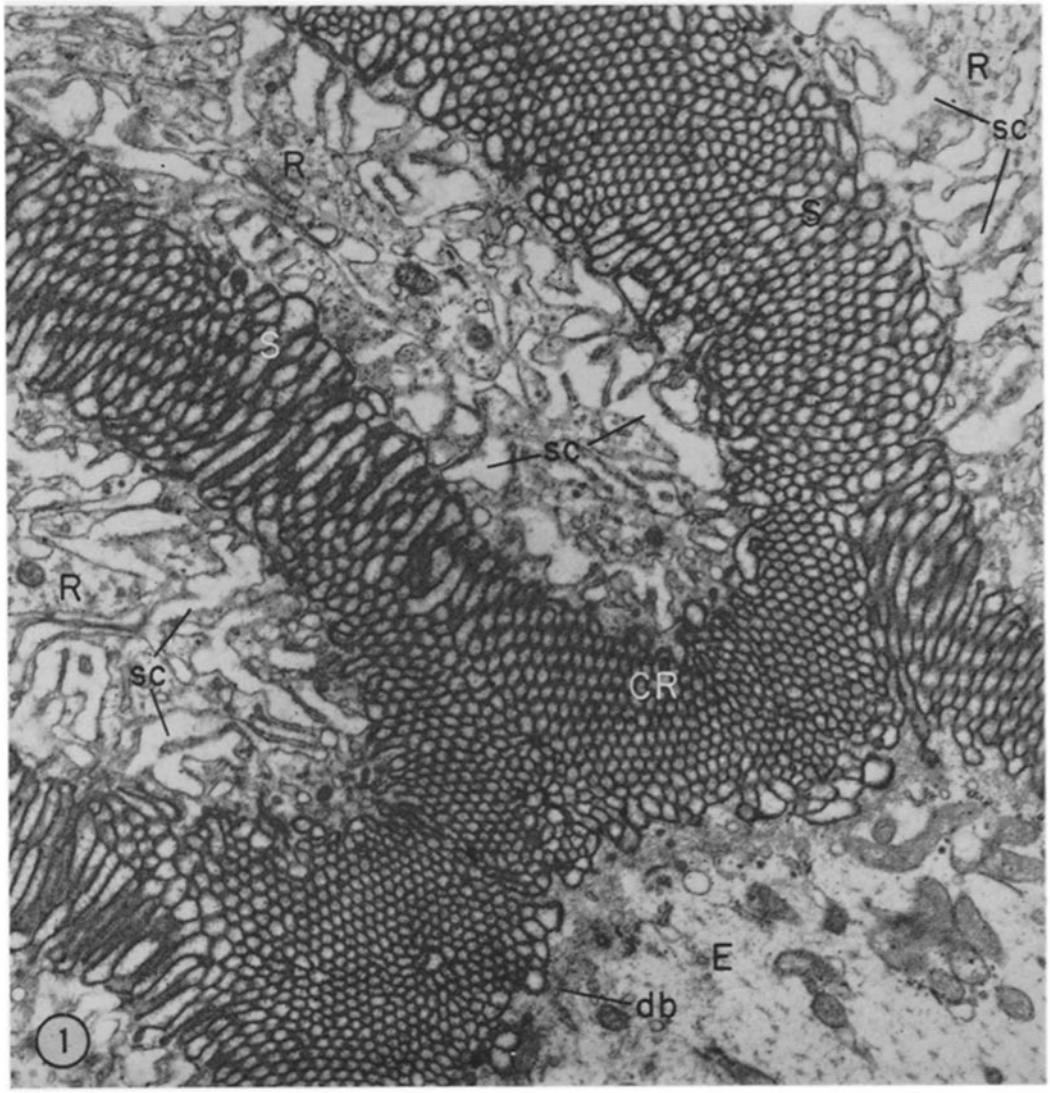


FIGURE 1 Part of the central ring (*CR*) and of three spokes (*S*) of the rhabdom. The central portions of four retinular cells (*R*) are included also. Numerous subrhabdomere cisternae (*sc*) are observed on both sides of the spokes and at the outer border of the central ring. Subrhabdomere cisternae are not present within the distal process of the eccentric cell (*E*). In their place a band of dense material (*db*) is found. × 17,000.

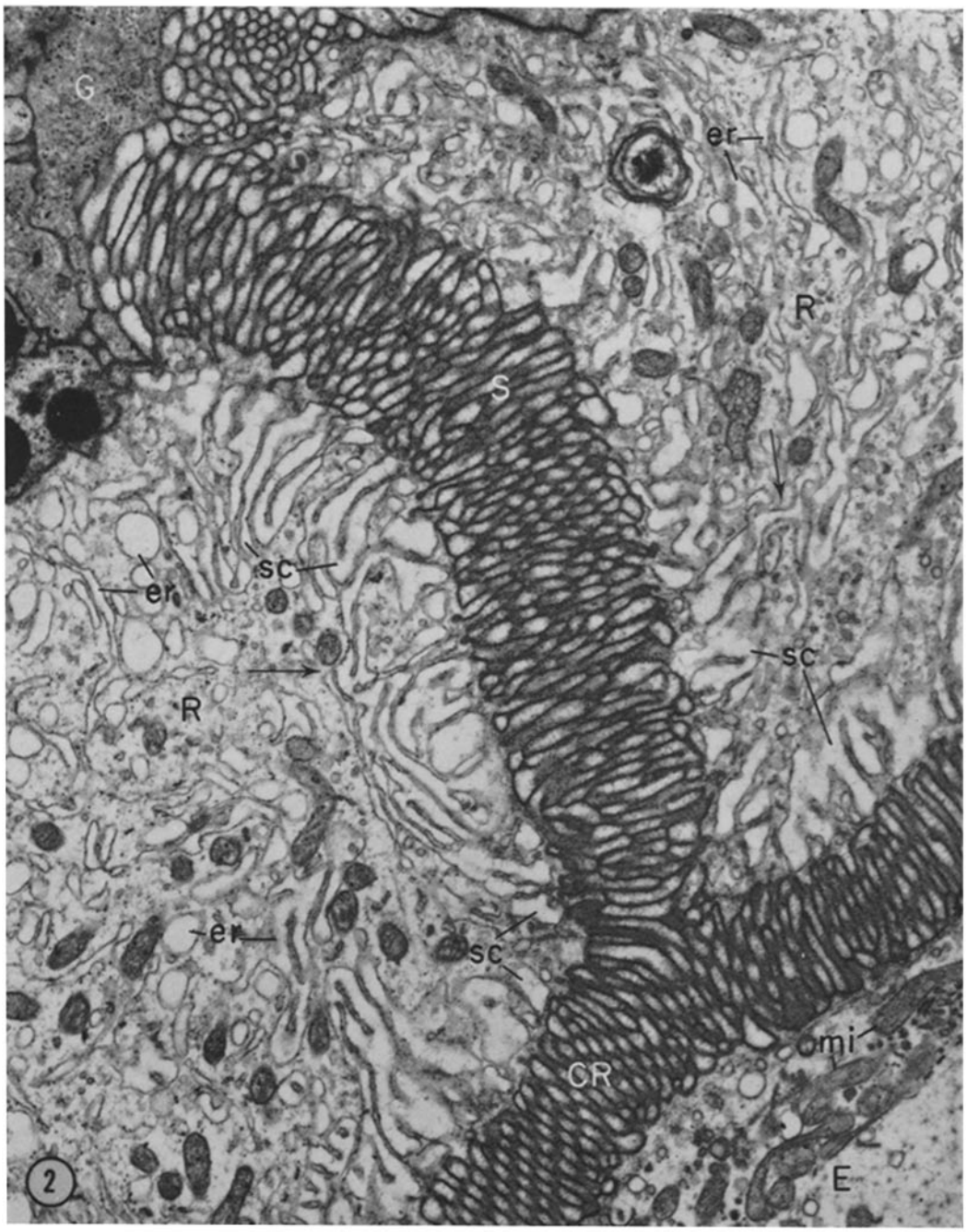


FIGURE 2 A spoke of the rhabdom (*S*) is shown extending from the central ring (*CR*) to the end of a glial septum (*G*). The endoplasmic reticulum (*er*) in the retinular cells (*R*) on each side is very conspicuous. Few ribosomes are attached to the endoplasmic reticulum membranes. Subrhabdomere cisternae (*sc*) underlie both borders of the spoke and the retinular cell side of the central ring. At the arrows the subrhabdomere cisternae are seen reaching deeper areas of the retinular cell cytoplasm. Within the distal process of the eccentric cell (*E*) subrhabdomere cisternae are not found. Mitochondria (*mi*) are clustered near the surface of the distal process. × 17,000.

But, before the cell junctions can be described, it will be necessary to give some consideration to the structure of the ommatidial cells, particularly to the cytological features relevant to the central problem of intercellular relationships.

Structure of the Ommatidial Cells

RETINULAR CELLS: The dominant characteristics of retinular cells are the abundance and the development of the endoplasmic reticulum (Figs. 1, 2 and 4). Numerous expanded or flattened cisternae, present throughout the retinular cell cytoplasm, give the cell a characteristic spongelike appearance. Endoplasmic reticulum near the cell surface can be differentiated into two types: one at the nonrhabdomere surface and one at the rhabdomere surface. At the nonrhabdomere surface of the retinular cell, endoplasmic reticulum cisternae are very frequently seen approaching the cell membrane and following a parallel course for a distance of 0.5–1 μ. The interval between the cell and cisternal membranes is approximately 150 A (Figs. 4, 15, and 16). The arrangement of these cisternae is similar to that of the subsurface cisternae described by Rosenbluth (32) in rat neurons. In retinular cells, however, subsurface cisternae are not always flattened (Figs. 4 and 5).

At the rhabdomere surface an uninterrupted series of expanded cisternae underlies the origins of the microvilli (Figs. 1, 2, 6 and 12). The proximity of these cisternae to the rhabdomere suggests that they are a special form of subsurface cisternae; this similarity will be stressed by terming them subrhabdomere cisternae. The distance separating the subrhabdomere cisternae from the cell membrane is usually somewhat larger and less regular than the distance between the cell membrane and the subsurface cisternae (Figs. 12 and 14). Occasionally both types of cisternae are seen communicating with one another (Fig. 5). Very few ribosomes are found attached to the endoplasmic reticulum membranes (Figs. 2 and 4), and even fewer on the subsurface and subrhabdomere cisternae (Figs. 4 and 12).

The abundant and closely packed endoplasmic

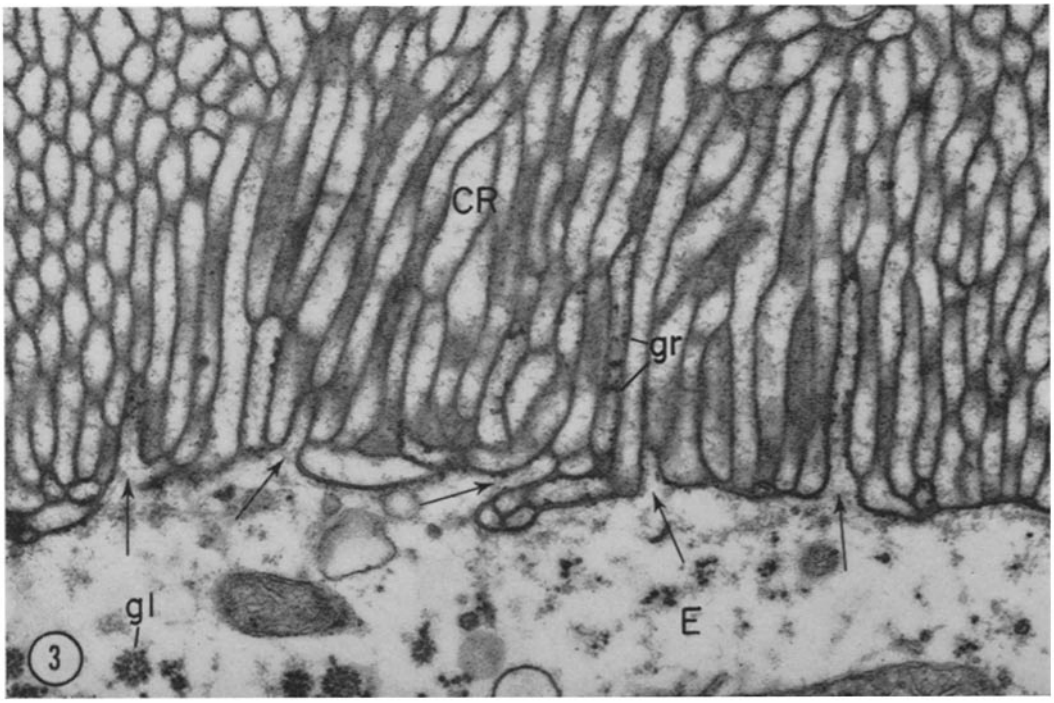


FIGURE 3 Part of the central ring of the rhabdom (*CR*) and of the distal process of the eccentric cell (*E*). Several microvilli originate from the eccentric cell (arrows) and become part of the rhabdom. Granules grouped in rosettes (*gl*), probably representing glycogen, are seen within the eccentric cell. Granules of another type (*gr*, see text) are attached to the membranes of some microvilli. × 34,000.

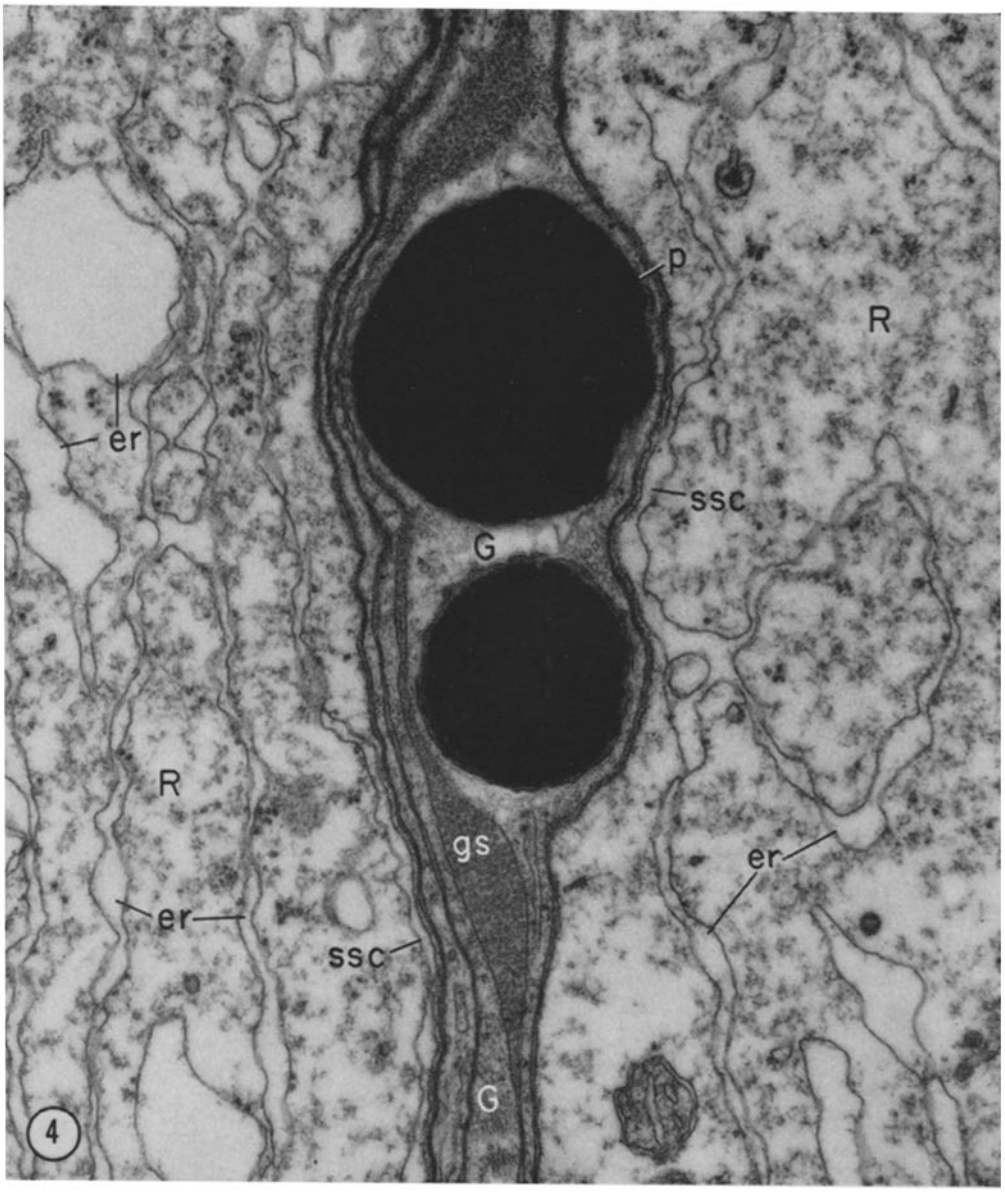


FIGURE 4 Segment of a glial septum extending radially between two retinular cells (*R*). The septum is formed in this case by three glial processes (*G*) embedded in a granular ground substance (*gs*). Two large pigment granules (*p*) are present in one of the glial processes. The endoplasmic reticulum (*er*) of the retinular cells is connected to subsurface cisternae (*ssc*). Very few ribosomes are attached to the endoplasmic reticulum membranes and almost none to the subsurface cisternae. Stained in block with uranyl acetate. × 34,000.

reticulum cisternae might represent an intercommunicating system (24), but no evidence of this is available. That the subrhabdomere cisternae often extend deeply into the cytoplasm (Fig. 2) suggests continuity with other components of the endoplasmic reticulum. The same suggestion applies to the subsurface cisternae (Figs. 4 and 5). On the other hand, continuity or direct con-

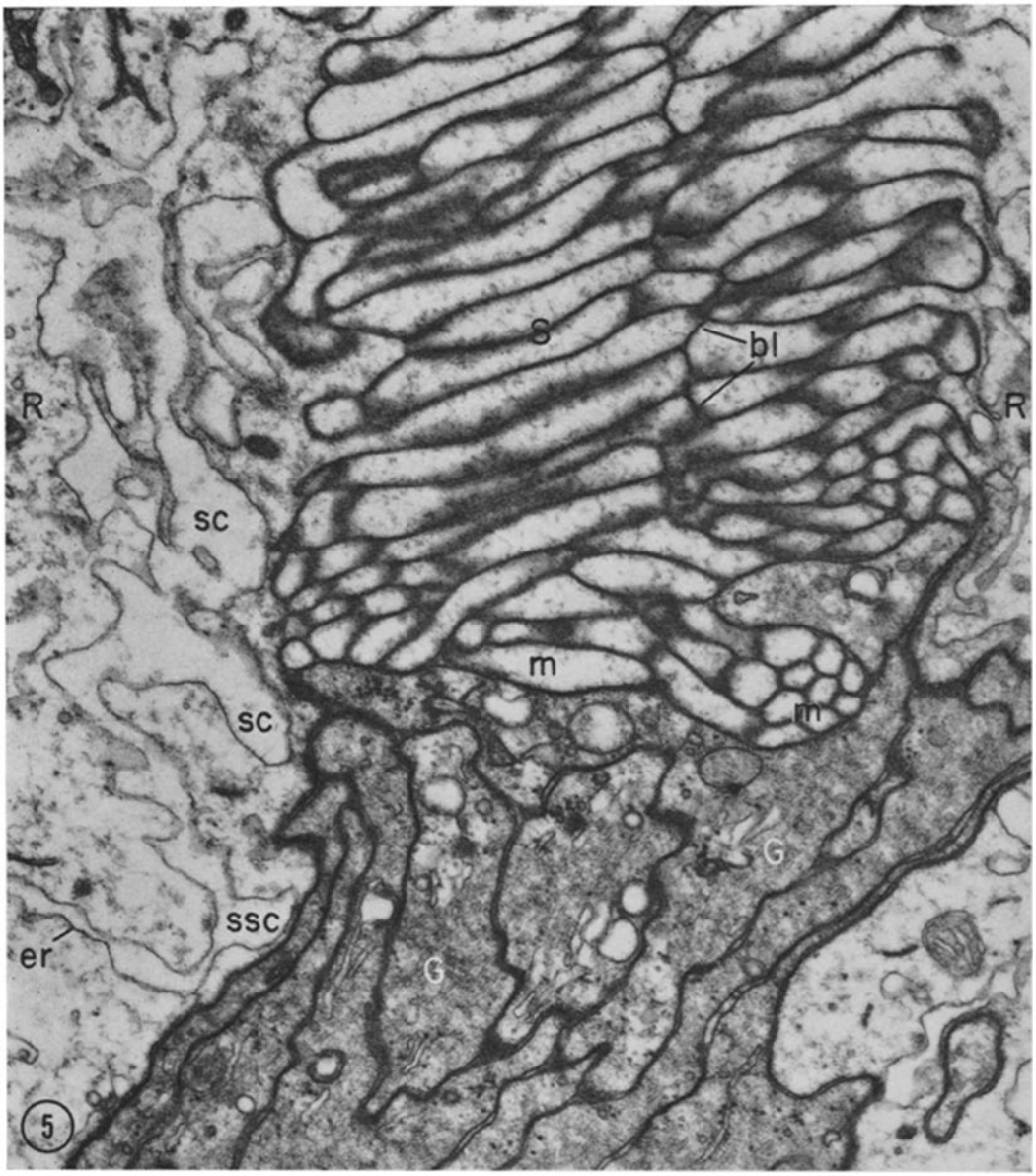


FIGURE 5 Central end of a glial septum between retinular cells and peripheral end of a spoke (*S*) of the rhabdom. The profiles of several glial processes (*G*) are seen within the septum and their close relationship with the microvilli (*m*) in the spoke is apparent. A tortuous line (*bl*) in the center of the spoke marks the boundary between the rhabdomeres of the adjacent retinular cells (*R*). The subrhabdomere cisternae (*sc*) on the left are continuous with a sub-surface cisterna (*ssc*) which in turn is connected to the deeper endoplasmic reticulum (*er*). Stained in block with uranyl acetate. × 34,000.

tact between either subrhabdomere or subsurface cisternae and the cell membrane has not been observed.

ECCENTRIC CELLS: In conspicuous contrast with retinular cells, the eccentric cell lacks subrhabdomere cisternae (Figs. 1–3, 6, and 13; see however Fig. 12). The cell cytoplasms on each side of the central ring[1] of the rhabdom are

[1] The expression *central ring* will be used to refer to the two-dimensional appearance of the core of the

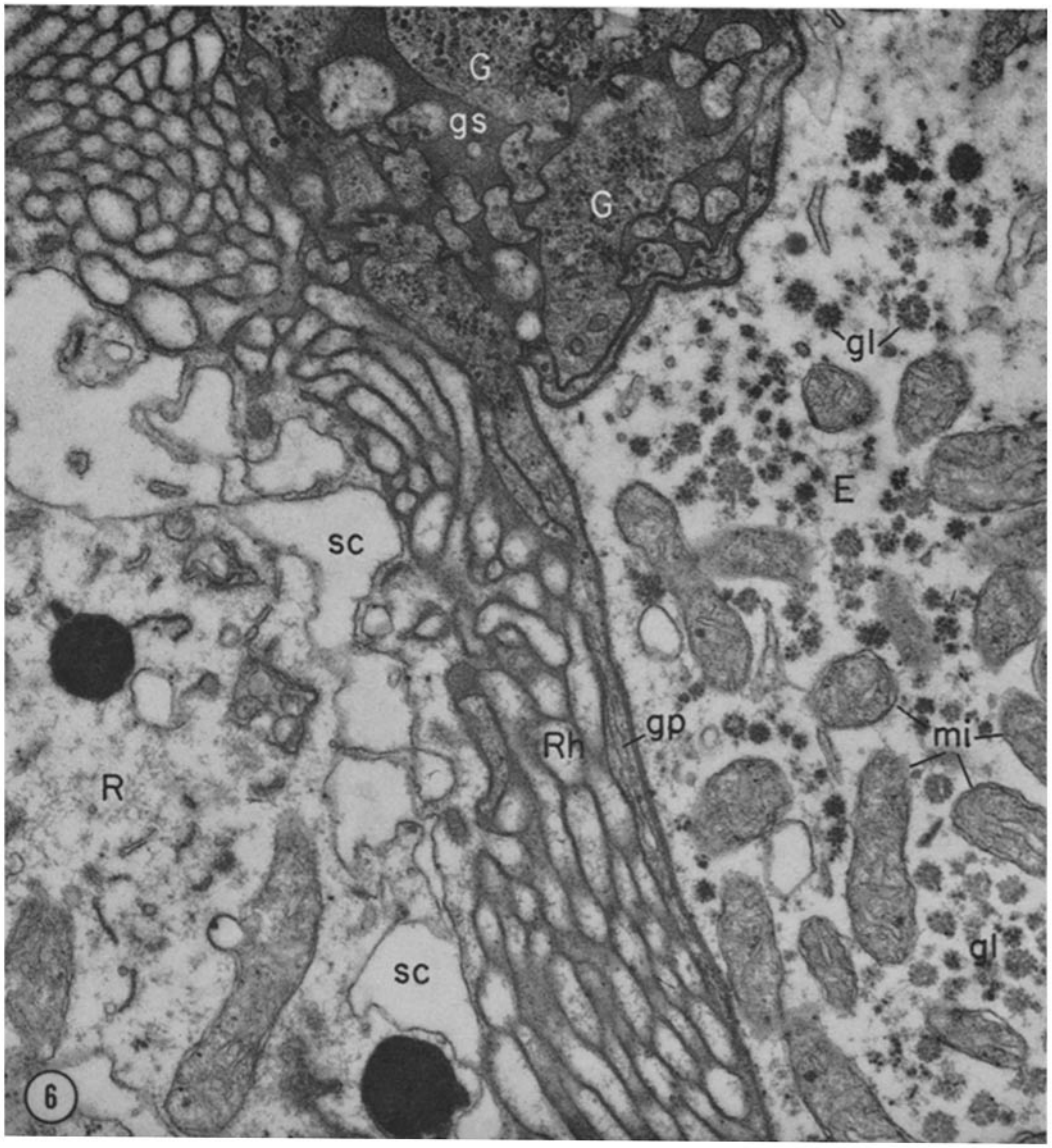


FIGURE 6 Glial processes (*G*) intervening between a retinular cell (*R*) and the origin (horizontal portion) of the distal process of the eccentric cell (*E*). The glial processes are embedded in a granular ground substance (*gs*). Towards the center of the ommatidium only a thin glial prolongation (*gp*) extends between the eccentric cell and the rhabdomere of the retinular cell (*Rh*). Even at this level the retinular cell side is marked by the presence of subrhabdomere cisternae (*sc*) closely related to the microvilli. Mitochondria (*mi*) and granular rosettes (*gl*) are abundant in the eccentric cell. Numerous dense granules are also evident within the glial processes. × 34,000.

clearly distinct from one another: the eccentric cell surface is devoid of the row of large membrane-limited spaces which characterize the boundary between the retinular cell and the rhabdom (Figs. 1, 2, 6, and 12). The spokes,[1] however, are symmetrical (Figs. 1 and 2). Subsurface cisternae are not absent from the eccentric cell. They can be seen frequently at the cell body,

rhabdom. Similarly, the sections of the finlike radial projections of the rhabdom will be referred to as *spokes*.

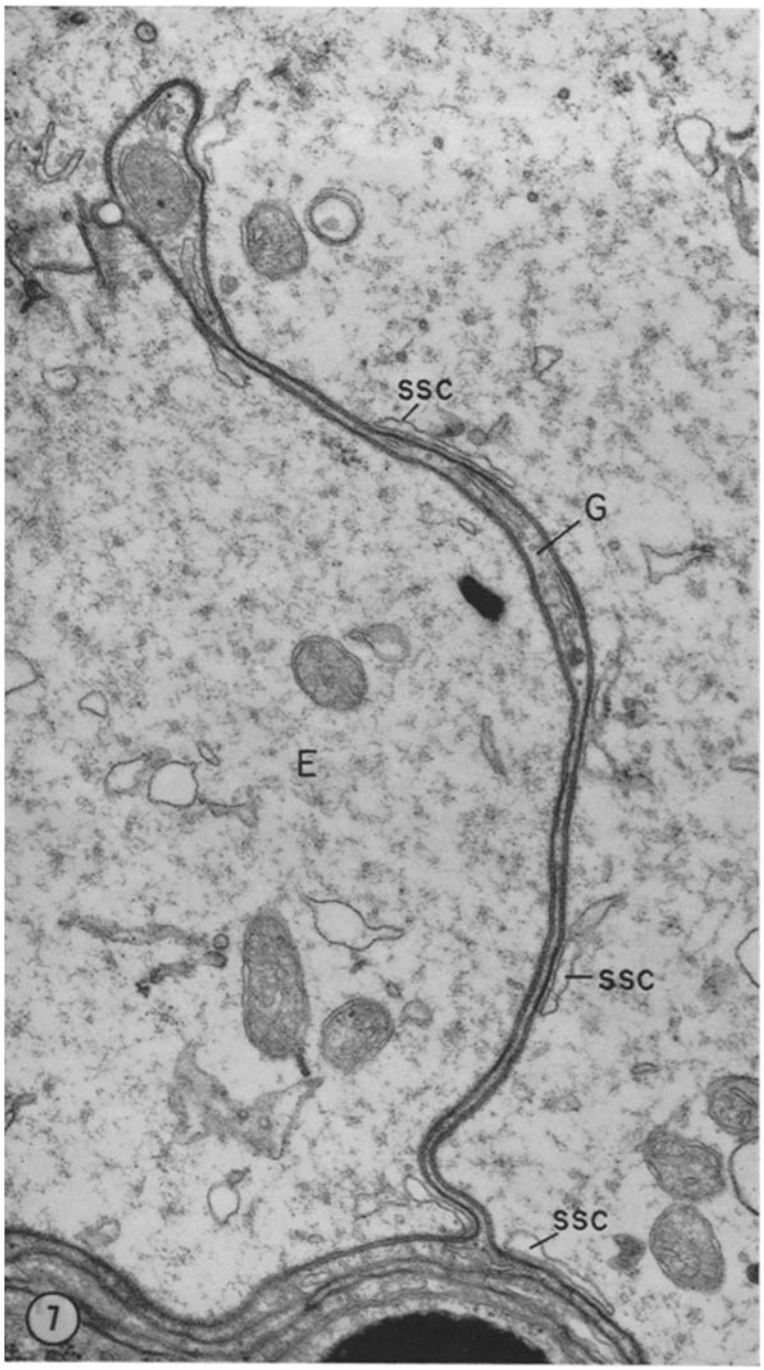


FIGURE 7 Glial evagination (*G*) penetrating within an infolding of the plasma membrane of the eccentric cell (*E*). Several subsurface cisternae (*ssc*) are seen subjacent to the eccentric cell membrane. Stained in block with uranyl acetate. × 34,000.

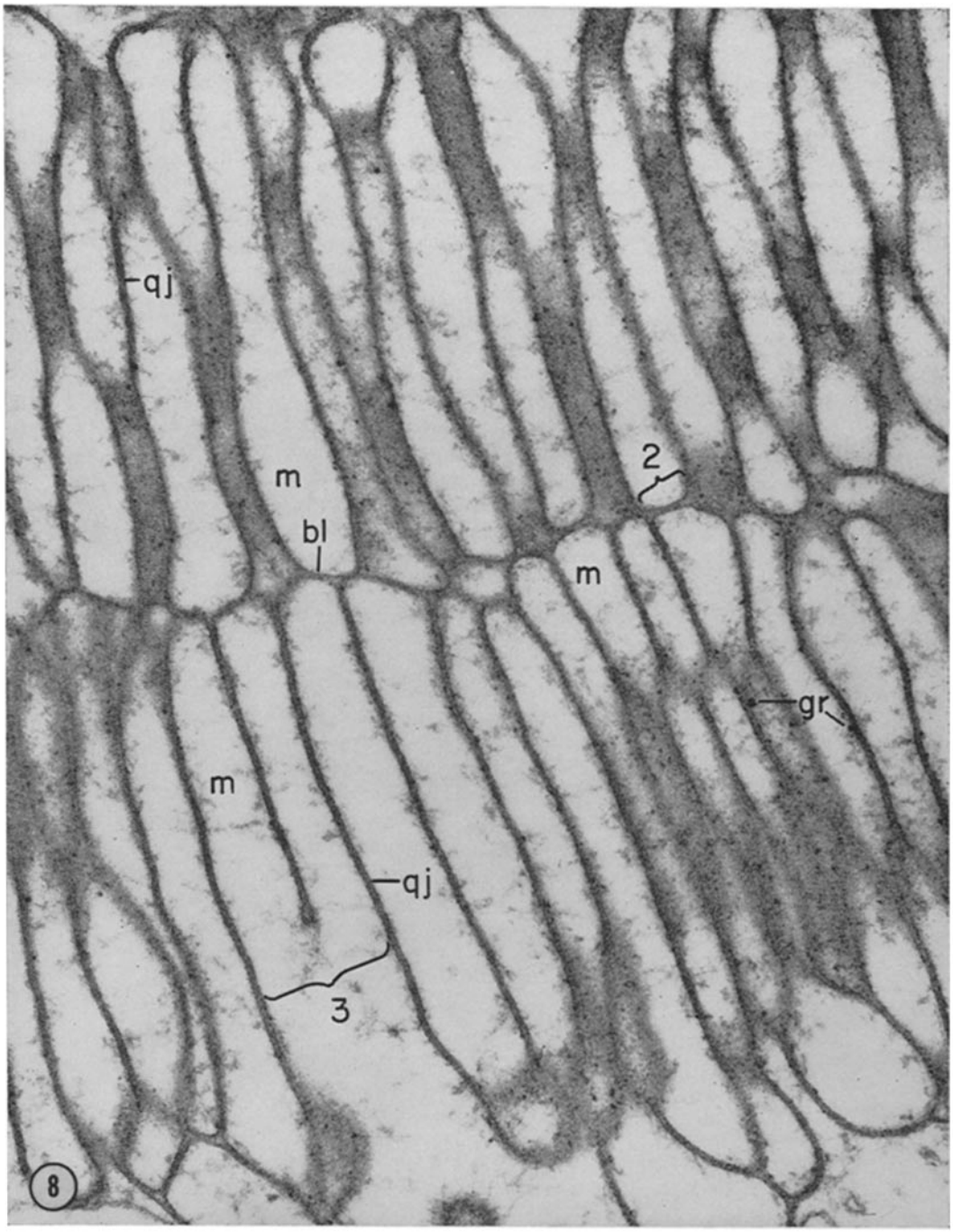


FIGURE 8 Segment of a spoke of the rhabdom. The uninterrupted quintuple-layered junctions (*qj*) between adjoining microvilli (*m*) of the retinular cells are shown. The zigzagging line (*bl*) between the two series of microvilli represents the boundary between the adjacent retinular cells. Dense granules (*gr*) are related closely to the junctional membranes. (Figs. 9 and 10 show enlargements of areas *2* and *3*). × 70,000.

where the plasma membrane is adjacent to the surface of glial cells (Fig. 7). However, the rest of the endoplasmic reticulum does not exhibit the extensive development found in retinular cells.

The membrane of the distal process of the eccentric cell is in intimate contact with the rhabdom (*vide infra*). As first shown by Miller (20), the rhabdom is formed by an array of microvilli originating from the surface of the visual cells; it has been thought, since then, that all the microvilli in the rhabdom originate from retinular cells. Nevertheless, examination of the boundaries of the distal process reveals that the eccentric cell also contributes microvilli to the central ring of the rhabdom (Figs. 3, 12, and 13). Therefore, although the spokes of the rhabdom are formed exclusively by the rhabdomeres of the retinular cells, microvilli from both eccentric and retinular cells are present at the central ring. Since the two types of microvilli in the central ring are identical (Fig. 13), they can only be identified if the connection with the cell of origin can be traced. In the case of the eccentric cell this has proved a rather difficult task because it is only infrequently that several of these connections can be seen in any particular field (Fig. 3). When the retinular cell border is inspected, however, direct continuity between the cytoplasm and the core of the microvilli is found in many instances (Fig. 12). This observation indicates that in any given segment of the central ring of the rhabdom the microvilli of the retinular cells outnumber those from the eccentric cell. The absolute contribution of the eccentric cell to the rhabdom is, however, not negligible, considering that microvilli originate throughout the perimeter of the distal process.

Underlying the surface of the distal process, a discontinuous, dense band is observed (Figs. 1 and 12) which, owing to its association with the microvilli, has a certain resemblance to the terminal web in the apical border of intestinal epithelial cells (23). Numerous rosettes of granules, probably representing glycogen (18, 26), are observed within the soma and the distal process of the eccentric cell (Figs. 3 and 6). Similar granules are also found in the retinular cells. Mitochondria, which are abundant in both cells (Figs. 2 and 6), are clustered near the surface of the distal process of the eccentric cell (Fig. 2).

GLIAL CELLS: These cells, the third type of ommatidial cell, have received little attention in the past. They are flattened cells that completely ensheathe the periphery of the ommatidium and also penetrate it to intervene between contiguous visual cells. Miller (20) referred to glial cells as pigment cells, but since pigment granules are also abundant within the visual cells, they cannot be taken as a basis for specific identification. Watase (37) discussed at some length the sheath cells, which he termed epithelial cells because they are nonneuronal ectodermal derivatives. In modern terminology these cells correspond to glial cells and their relationship to the neuronal elements in the ommatidium seems to be the best justification for this view.

Retinular cells are always separated from one another by a variable number of glial cells embedded in a granular ground substance (Fig. 4). These glial processes form septa that extend radially from the periphery of the ommatidium to the rhabdomere of the retinular cells. At this point the glial cell processes are closely related to the retinular cell microvilli (Figs. 2 and 5). Glial cells also intervene between the bodies of the eccentric and retinular cells, with a thin glial process always separating the horizontal portion of the distal process from the retinular cell microvilli (Fig. 6). Glial prolongations are also found protruding within the body of the visual cells. These are long and slender glial sheets with a broadened distal edge that penetrate within invaginations of the plasmalemma of the visual cells (Fig. 7). This type of glial process is encountered more frequently in the eccentric cell than in retinular cells. Entirely similar evaginations of the glial cells have been reported in cockroach ganglia by Hess (14) who interpreted them as the trophosphongium of Holmgren (15).

Cell Junctions

JUNCTIONS BETWEEN RETINULAR CELLS: These junctions are found within the spokes of the rhabdom. The tips of the microvilli originating from contiguous retinular cells meet at the center of the spokes (20). In sections parallel to the axis of the microvilli the cell limits are seen as a tortuous line separating the two series of microvilli (Fig. 8). When this boundary is examined, it is found to exhibit the typical stratified appearance (Fig. 9) that characterizes the apposition or fusion of adjacent cell membranes in a variety of tissues (4, 7, 12, 16, 22, 28). From the several terms that have been proposed to designate this structural arrangement, the expression quintuple-

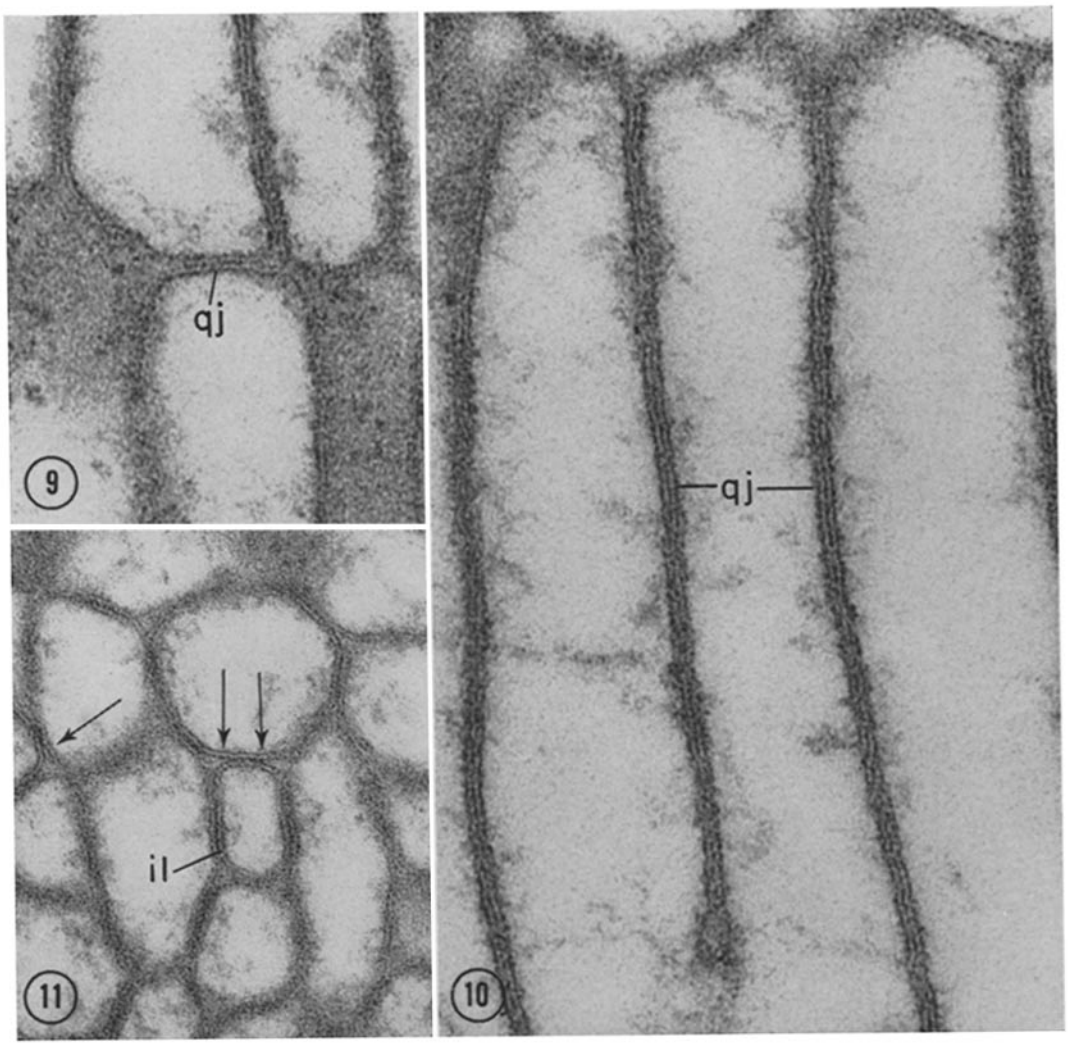


FIGURE 9 Higher magnification of area *2* in Fig. 8. At the center line of the spoke a quintuple-layered junction (*qj*) is observed between the tips of microvilli originating from each of the contiguous retinular cells. × 176,000.

FIGURE 10 Higher magnification of area *3* in Fig. 8. The quintuple-layered junctions (*qj*) in the spoke include three dense layers of approximately equal thickness. × 176,000.

FIGURE 11 Cross-sections of microvilli in a spoke. The intermediate layer (*il*) in the quintuple-layered junctions appears denser and thicker than each of the inner leaflets of the cell membranes. At points where three microvilli meet, the outer leaflets of the cell membranes separate from one another (arrows). Stained in block with uranyl acetate. × 141,000.

layered junction (16, 22) will be chosen here because its generality and descriptive value seem appropriate in this particular instance. The center line of the spokes can, therefore, be defined as a series of quintuple-layered junctions between the tips of the microvilli of adjacent retinular cells (Fig. 9). The lines of demarcation between the sides of adjoining microvilli of a single retinular cell are also quintuple-layered junctions (Fig. 10). Since the intermediate dense layer of each quin-

tuple-layered junction represents the apposition of the outer leaflets of the contributing cell membranes, there is no demonstrable extracellular space within the spokes of the rhabdom except perhaps for narrow interstices at points where three microvilli are in contact (Fig. 11).

When only sections are stained with uranyl and lead, the intermediate dense layer in the quintuple-layered junctions is as dense and thick as each of the inner leaflets of the contributing cell membranes (Figs. 9 and 10). After staining in block with uranyl, however, this layer appears even denser and is approximately twice as thick as each inner leaflet (Fig. 11). The total thickness of the quintuple-layered junctions (~150 A) is approximately equivalent to the sum of the thicknesses of the contributing membranes. These membranes seem, therefore, to be merely in contact without any loss of substance. Since measurements by eye are rather arbitrary (30), this conclusion is expressed only under such reservations.

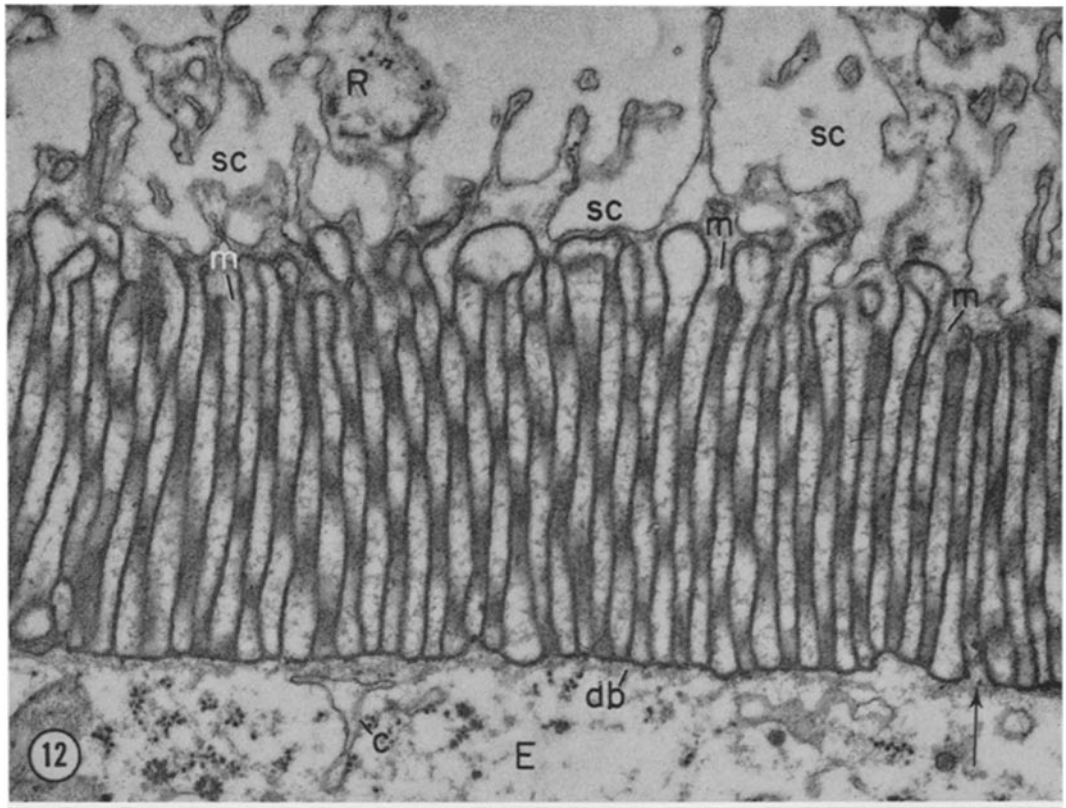
JUNCTIONS BETWEEN ECCENTRIC AND RETINULAR CELLS: These are found at the central ring of the rhabdom. In sections parallel to the axis of the microvilli the central ring does not show a dividing line at the center (Fig. 12). This indicates that the microvilli from the eccentric and retinular cells interdigitate instead of meeting end-on. The fact that a great majority of the microvilli originate at the retinular cell side also accounts for this appearance. The lines of demarcation between the sides of the microvilli are quintuple-layered junctions as in the spokes (Fig. 14), but in this instance a number of them are of an intercellular nature (Fig. 13). Most of the intercellular junctions occur, however, between the tips of the retinular cell microvilli and the plasma membrane of the distal process (Figs. 13 and 14). The boundary between the distal process of the eccentric cell and the rhabdom represents, therefore, an extensive quintuple-layered junction. In material stained in block with uranyl the intermediate dense layer of the quintuple-layered junctions is thicker than each of the inner leaflets of the cell membranes (Fig. 14). This appearance, as well as the total thickness of the junctions, is identical with that found in the spokes, and the same comments apply.

Dense granules, 100–200 A in diameter, are frequently found in the rhabdom, both in the central ring (Figs. 3 and 13) and in the spokes (Fig. 8). These granules occasionally show a less dense core which gives them a vesicular appearance. They are usually closely related to the membranes of the microvilli (Fig. 13). The significance or chemical nature of the granules is entirely unknown.

JUNCTIONS BETWEEN GLIAL AND VISUAL CELLS: Quintuple-layered junctions between retinular and glial cells are abundant on the lateral surfaces of the retinular cells, where they are related to the glial septa (Fig. 15). These junctions resemble those in the rhabdom in exhibiting a very dense and thick intermediate layer after staining in block with uranyl acetate (Fig. 15). Subsurface cisternae are found, in a great number of instances, underlying the junctions (Fig. 15). This association is so common that it is difficult to avoid the interpretation that the cisterna and the junction constitute a morphological unit. However, at the peripheral surface of the

FIGURE 12 Segment of the central ring of the rhabdom. The constituent microvilli are parallel to the plane of section and there is no dividing line at the middle. The retinular cell side (*R*) is indicated by the subrhabdomere cisternae (*sc*). The continuity of the retinular cell cytoplasm with the core of numerous microvilli is easily detected (*m*). On the eccentric cell side (*E*) such continuity can be clearly seen only at the point marked by an arrow. A band of dense material (*db*) underlies the surface of the distal process. The adjacent cisterna (*c*) is an exceptional finding and would seem to represent the eccentric cell equivalent of the subrhabdomere cisternae. × 34,000.

FIGURE 13 Part of the central ring (*CR*) of the rhabdom and surface of the distal process of the eccentric cell (*E*). The arrows point to quintuple-layered junctions between a microvillus (*m*) originating from the eccentric cell and other microvilli which presumably belong to a retinular cell. The boundary between the rhabdom and the eccentric cell is also a quintuple-layered junction (*qj*). Dense granules (*gr*) are associated closely with the membranes of the microvilli. × 70,000.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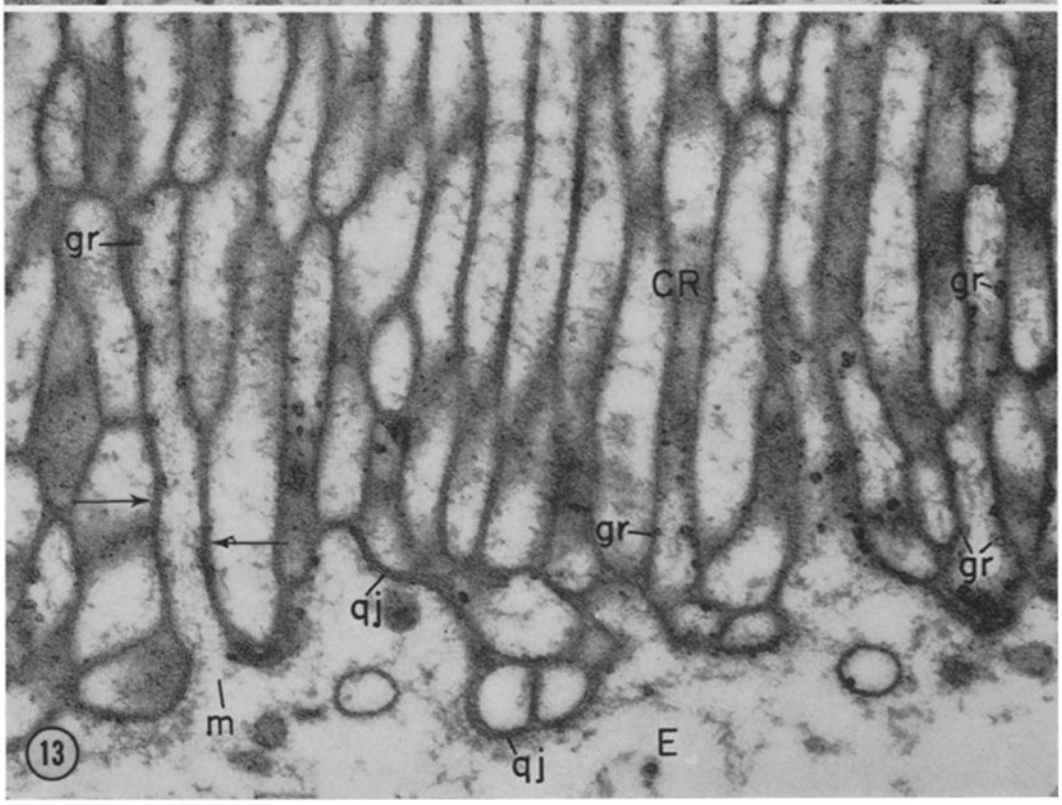
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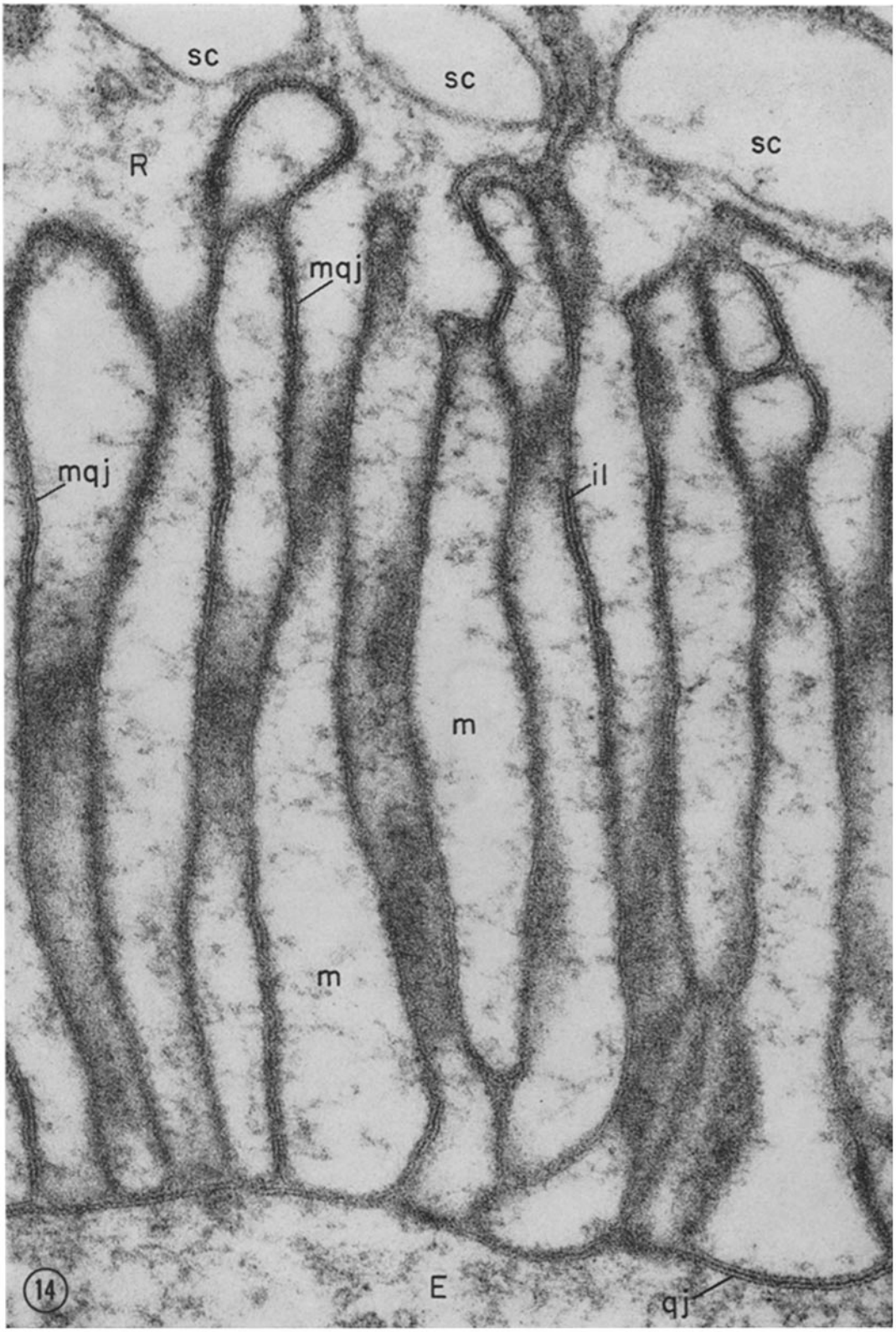
retinular cells, quintuple-layered junctions with glial cells are less often encountered. Here it is possible to find subsurface cisternae related to areas of the retinular cell membrane exposed to extracellular space (Fig. 16). Possibly the association of quintuple-layered junctions and subsurface cisternae is only incidental; it might reflect the fact that both structures are very numerous on the lateral surfaces of retinular cells. Quintuple-layered junctions with glial cells are observed even at the microvilli of the retinular cells (Fig. 17). This is a constant finding at the areas of contact between the glial septa and the spokes of the rhabdom. These junctions do not differ noticeably from those between the neighboring microvilli (Fig. 17). Quintuple-layered junctions between eccentric and glial cells are also common both at the surface of the eccentric cell (Fig. 17) and in relation with the glial processes invaginating the plasma membrane (Fig. 18). Finally, quintuple-layered junctions are also found between contiguous glial processes (Fig. 15).

DISCUSSION

STRUCTURE OF THE RETINULAR CELLS: Although little information is available on the fine structure of retinular cells in other compound eyes, it seems likely that the extensive development of the endoplasmic reticulum is not a specific feature of the *Limulus* retinula. In particular, structures which resemble the subrhabdomere cisternae can be occasionally detected in electron micrographs of already published work, although they do not seem to have attracted the attention of previous workers. Large vacuoles underlying the rhabdom of a crustacean eye are observed in electron micrographs obtained by Wolken and Gallik (41) but no mention of this is made in the text. On the other hand, Goldsmith (11) noted the presence of endoplasmic reticulum membranes adjacent to the rhabdom in the eye of the honeybee. The cavities surrounding the rhabdom in the eye of the grasshopper, which Fernández Morán (9) interpreted as tracheolar spaces, might also represent subrhabdomere cisternae. The presence of numerous vesicles in the cytoplasm of retinular cells of *Bombyx mori* has been mentioned by Eguchi (5) and many vacuoles are noticed in retinular cells of *Lycosa* (36) but again in this case no reference is made to them.

There is at present little basis for an understanding of the possible functional significance of the conspicuous endoplasmic reticulum in retinular cells of *Limulus*. The numerous cytoplasmic cisternae might represent a continuous system of channels linking the subrhabdomere cisternae with the subsurface cisternae. As recently shown by Borsellino et al. (2), one of the earliest detectable effects of light stimulation is an increase in the conductance of the nonrhabdomere surface of the retinular cell. It seems necessary, therefore, to consider the possibility that the endoplasmic reticulum might be involved in the spread of excitation away from the rhabdom. This would imply that the subrhabdomere and subsurface cisternae are functionally connected to the adjacent cell membrane, although no evidence of continuity or contact has been found. The possibility of such a type of interaction between the cell membrane and subsurface cisternae has been discussed by Rosenbluth (32) and his considerations are also applicable in the particular case of the retinular cell. The utilization of the endoplasmic reticulum for intracellular conduction (24) might be a way to circumvent the special problems introduced by the virtual absence of extracellular space in the rhabdom. The validity of this line of argument, however, becomes very questionable when it is considered that a great number of subsurface cisternae at the end of the hypothetical conduction system are related to quintuple-layered junctions with glial cells. These junctions could be interpreted as a device to promote metabolic or ionic exchanges (8) between retinular and glial cells. Also, the peculiar arrangement of the endoplasmic reticulum could similarly be interpreted as serving primarily the purpose of intracellular transport (24) from the junctions with glial cells to the rhabdom. This hypothesis at

FIGURE 14 Part of the central ring of the rhabdom. Subrhabdomere cisternae (*sc*) are seen on the retinular cell side (*R*). The borderline between the distal process of the eccentric cell (*E*) and the rhabdom is a quintuple-layered junction (*qj*). Between the microvilli (*m*) quintuple-layered junctions are also evident (*mqj*). The intermediate dense layer (*il*) in all the quintuple-layered junctions is thicker than the inner leaflets of the cell membranes. Stained in block with uranyl acetate. × 141,000.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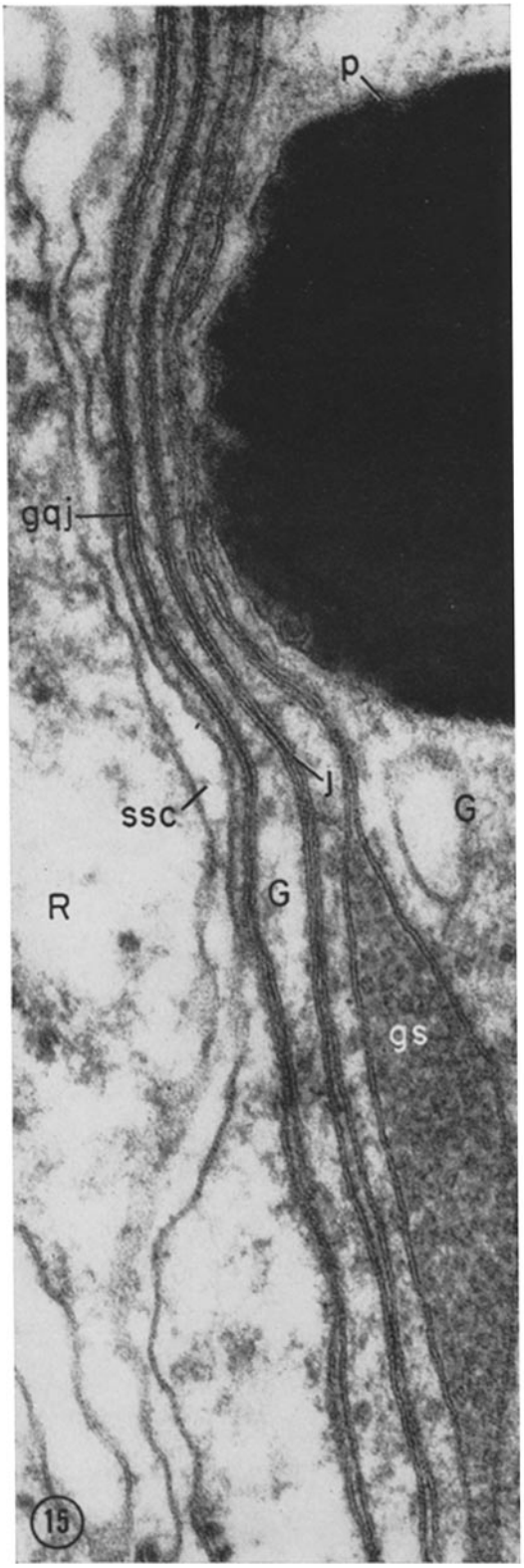

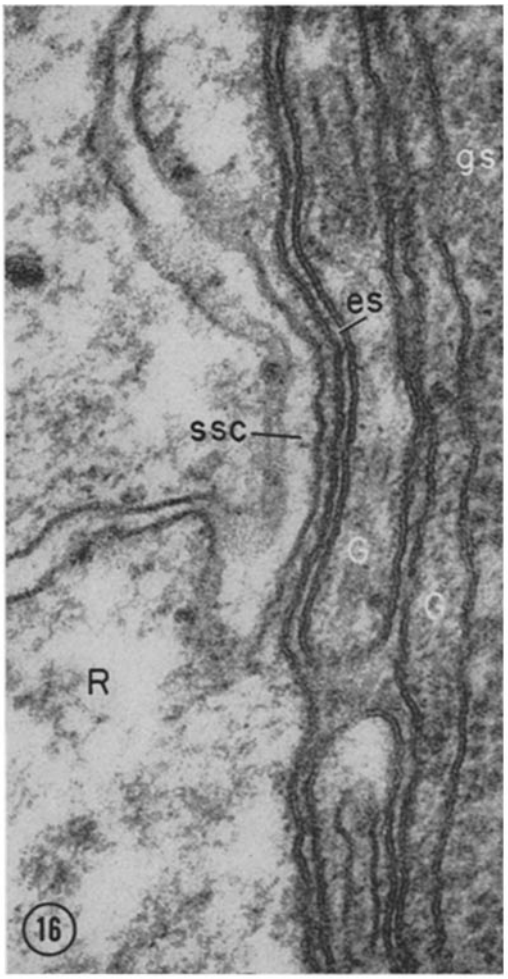

FIGURE 16 Boundary between a retinular cell (*R*) and glial processes (*G*) at the periphery of the ommatidium. A sub-surface cisterna (*ssc*) underlies an area of the retinular cell membrane exposed to extracellular space (*es*). *gs*, ground substance. Stained in block with uranyl acetate. × 141,000.

FIGURE 15 Boundary between a retinular cell (*R*) and a glial septum. Area similar to that shown in Fig. 4. The septum is formed by several glial processes (*G*) related at some points by quintuple-layered junctions (*j*). A quintuple-layered junction is also seen occluding the space between a glial process and the retinular cell (*gqj*). A subsurface cisterna (*ssc*) is adjacent to this quintuple-layered junction. *p*, pigment granule; *gs*, intercellular ground substance. Stained in block with uranyl acetate. × 141,000.

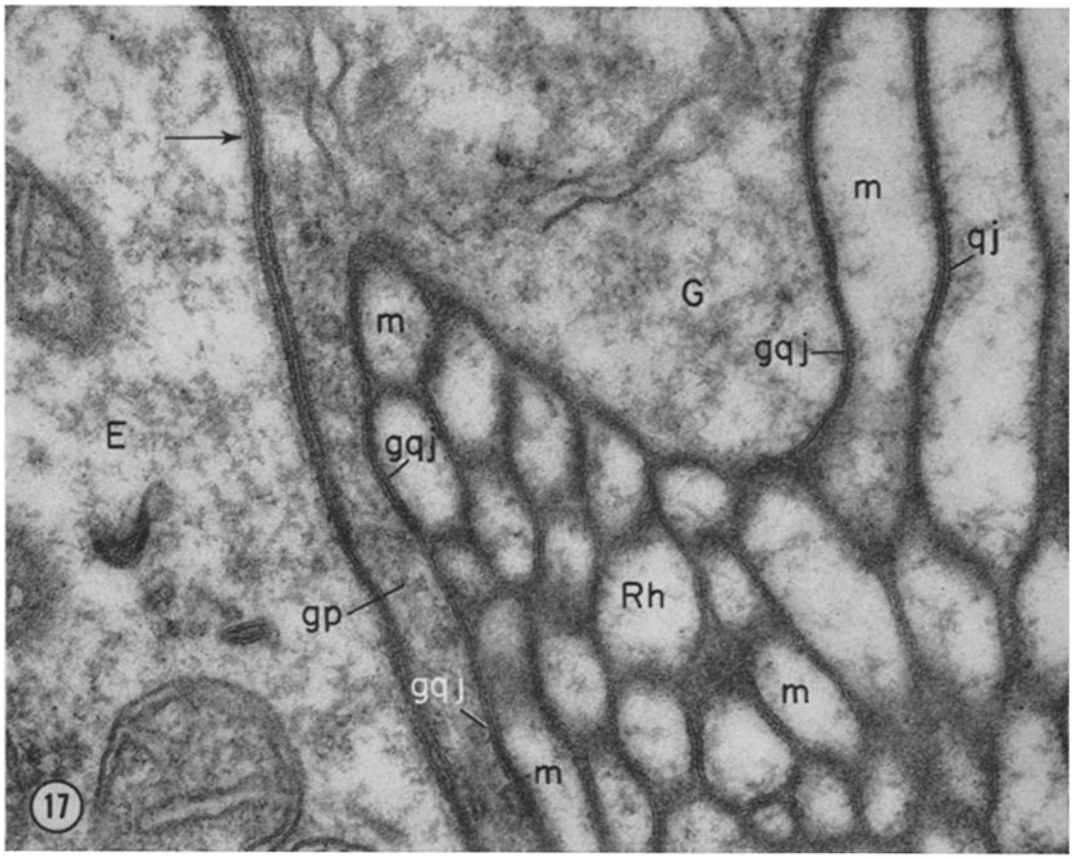

FIGURE 17 Area similar to that in Fig. 6. A glial cell (*G*) extends a thin prolongation (*gp*) between the eccentric cell (*E*) and the rhabdomere of a retinular cell (*Rh*). Quintuple-layered junctions (*gqj*) are established between the glial cell and the microvilli (*m*) of the retinular cell. These junctions are entirely similar to the quintuple-layered junctions between adjoining microvilli (*qj*). At the arrow a quintuple-layered junction between the eccentric and glial cell is seen with some difficulty due to oblique sectioning. Stained in block with uranyl acetate. × 105,000.

least has the merit of not requiring the additional assumption that the endoplasmic reticulum is a stable continuum.

STRUCTURE OF THE ECCENTRIC CELL: The observation that the surface of the distal process provides microvilli which contribute to form the central part of the rhabdom, suggests that the eccentric cell is a photoreceptor. It can at least be said to have the same claim to this denomination as the retinular cells. The generalized assumption that the rhabdom represents a photoreceptor structure receives support from the finding that the appearance of electrical responses in the compound eye parallels the development of the rhabdom (6). The eccentric cell in *Limulus* ommatidia has been occasionally regarded as a second order neuron, mainly due to its bipolar appearance and the failure to detect a rhabdomere structure on the surface of the distal process. Watase (37) described it as a ganglion cell. Waterman and Wiersma (38) discussed the possibility that the eccentric cell is a photoreceptor unit or a secondary neuron and were inclined to support the latter alternative. Miller (20) seems to have taken a similar position by referring to the eccentric cell as a bipolar neuron, although strictly speaking this term would not exclude a photoreceptor function. In at least one case, however, the existence of a rhabdomere in eccentric cells has been noted, namely by Eguchi (5) for the compound eye of *Bombyx mori*. This finding, however, was thought to place the eccentric cell in *Bombyx mori* in a

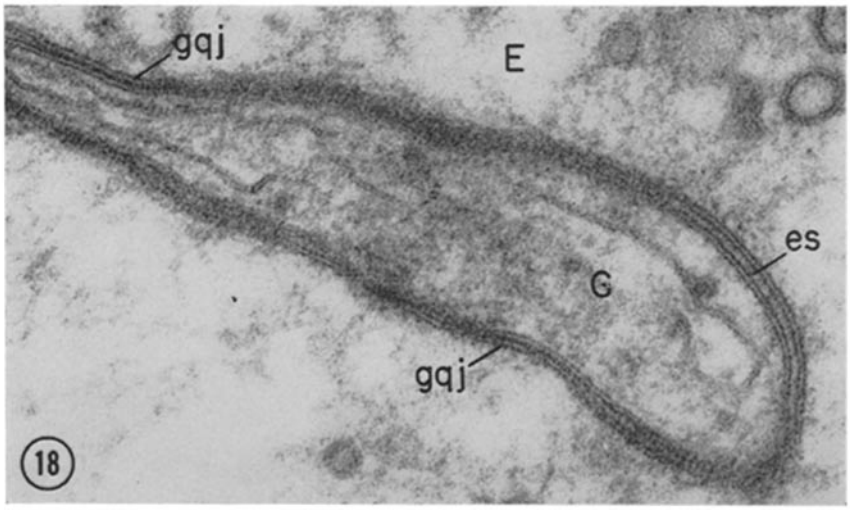

FIGURE 18 Broadened edge of a glial sheet (*G*) invaginating the membrane of the eccentric cell (*E*). Although some intercellular space is observed (*es*), areas of occlusion by quintuple-layered junctions (*gqj*) are also found. Stained in block with uranyl acetate. × 141,000.

different category from the eccentric cell in ommatidia of *Limulus*. The present findings suggest that both these eccentric cells may be essentially similar.

JUNCTIONS BETWEEN VISUAL CELLS: As shown above, these junctions occur in the rhabdom at the line of demarcation between adjoining microvilli. These contacts form quintuple-layered junctions whether the microvilli originate from a single retinular cell, from two adjacent retinular cells, or from a retinular cell and the eccentric cell. Furthermore, quintuple-layered junctions between the eccentric cell membrane and the tips of the microvilli of the retinular cells occur at the boundary between the distal process and the rhabdom. Analogous close contacts between microvilli have been reported in the octopus retina (21, 42). Moody and Robertson (21) interpreted their findings as indicating mere apposition of the adjoining membranes. As already mentioned, this also seems to be an adequate description of the junctions between visual cells in the ommatidium of *Limulus*.

The primary aim of this study was to investigate the structural basis for the electrical coupling between visual cells of *Limulus* (34). The finding of quintuple-layered junctions seems to provide such a basis since similar junctions have been shown to be associated with electrotonic transmission at electrical synapses (1, 4, 29, 31) and contacts between muscle cells (4, 16). Electrotonic junctions are sometimes referred to as areas of membrane fusion, but actual loss of substance or structural changes in the contributing membranes have as yet not been established (4, 30). Therefore, although the quintuple-layered junctions in the ommatidium of *Limulus* probably represent simple membrane contacts and not actual fusions, the available evidence is compatible with the assumption that the rhabdom provides an extensive electrotonic junction relating the retinular cells to one another and to the eccentric cell.

JUNCTIONS BETWEEN GLIAL AND VISUAL CELLS: The possibility that the function of these quintuple-layered junctions is to facilitate ionic and metabolic exchanges has been referred to above. This suggestion can not be given more serious consideration at present in view of the lack of physiological data indicating the operation of such cell to cell interaction in the living tissue. There are as yet no clearly defined morphological criteria which by themselves would suffice to assign proper value to quintuple-layered junctions in all the instances in which they are found in electron micrographs. A better knowledge on the function of glial cells in general will also be required in order to assess whether the quintuple-layered junctions between glial and visual cells represent a meaningful finding.

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