Z. Zellforsch. 108, 530—562 (1970)

The Fine Structure of the Retina of the Honey Bee Drone

An Electron Microscopical Study

ALAIN PERRELET*

Institute of Histology, University of Geneva School of Medicine, Geneva, Switzerland

Received March 9, 1970

Summary. The eye of the honey bee drone is composed of approximately 8,000 photoreceptive units or ommatidia, each topped by a crystalline cone and a corneal facet. An ommatidium contains 9 visual or retinula cells whose processes or axons pierce a basement membrane and enter the optic lobe underlying the sensory retina. The visual cells of the ommatidium are of unequal size: six are large and three, small. In the center of the ommatidium, the visual cells bear a brush of microvilli called rhabdomere. The rhabdome is a closed-type one and formed mainly by the rhabdomeres of the six large retinula cells. The rhabdomeric microvilli probably contain the photopigment (rhodopsin), whose modification by light lead to the receptor potential in the retinula cells. The cytoplasm of the retinula cells contains various organelles including pigment granules (ommochromes), and peculiar structures called the subrhabdomeric cisternae. The cisternae, probably composed of agranular endoplasmic reticulum undergo swelling during dark adaptation and appear in frequent connection with Golgi cisternae. Three types of pigment cells are associated with each ommatidium. The crystalline cone is entirely surrounded by two corneal pigment cells. The ommatidium, including its dioptric apparatus and corneal pigment cells, is surrounded by a sleeve of about 30 elongated cells called the outer pigment cells. These extend from the base of the corneal facet to the basement membrane. Near the basement membrane the center of the ommatidium is occupied by a basal pigment cell. Open extracellular channels are present between pigment cells as well as between retinula cells. Tight junctions within the ommatidium are restricted to the contact points between the rhabdomeric microvilli. These results are discussed in view of their functional implications in the drone vision, as well as in view of the data of comparative morphology.

Key-Words: Eye — Retina — Honey bee — Ultrastructure.

Résumé. L'oeil composé du faux-bourdon est formé d'environ 8000 unités photoréceptrices ou ommatidies. Chaque ommatidie, surmontée d'un appareil diotrique constitué d'une lentille cornéenne et d'un cône cristallinien, comporte 9 cellules visuelles dont les parties proximales (axones) pénètrent dans le lobe optique. Le lobe optique est séparé de la rétine sensorielle par une membrane basale. Les cellules visuelles formant l'ommatidie sont de taille inégale: six sont grandes et trois petites. Au centre de l'ommatidie, les grandes cellules visuelles forment de nombreuses microvillosités dont l'ensemble constitue le rhabdome. Celui-ci est du type fermé. La membrane des microvillosités contient probablement le photopigment. Le cytoplasme des cellules visuelles est riche en organites parmi lesquels des vacuoles allongées de réticulum endoplasmique lisse appelées citernes périrhabdominales. Les citernes changent de volume lors de l'adaptation à la lumière et à l'obscurité et apparaissent

* I am most indebted to Dr. Fritz Baumann, assistant professor in the Department of Physiology, University of Geneva School of Medicine, who contributed to this work with innumerable suggestions and precious advice. I also wish to thank Professors Ch. Rouiller and J. Posternak for constant support, and Mrs. A. Perrelet-Bridges for the correction of the English manuscript. Fig. 1 was done by R. Mira and the photographical work by Mrs. M. Sidler whose skilful assistance is gratefully acknowledged.

This work was supported by a grant from the "Fonds National Suisse de la Recherche Scientifique".

fréquemment en contact avec des complexes de Golgi ou des profiles de réticulum endoplasmique granulaire.

Trois types de cellules pigmentaires sont associés à l'ommatidie: les cellules pigmentaires du cristallin, les cellules pigmentaires externes, et la cellule pigmentaire basale. Les cellules pigmentaires du cristallin sont au nombre de deux et enveloppent le cône cristallinien. 27 à 30 cellules pigmentaires externes entourent l'ommatidie depuis la base de la cornée jusqu'à la membrane basale. La cellule pigmentaire basale occupe le centre de l'ommatidie lorsque les cellules visuelles se transforment en axones. Les divers types cellulaires de la rétine sont séparés les uns des autres par de minces espaces extracellulaires. Dans l'ommatidie, des jonctions serrées ne sont trouvées qu'entre les microvillosités rhabdomériques. Ces résultats sont discutés du point de vue de leur implication fonctionelle et de leur signification vis-à-vis de la morphologie comparée.

In his paper on the structure of the compound eye of the honey bee Phillips (1905) wrote: "The morphology of the compound eye has puzzled zoologists for years and much work has been done on the subject, but so diverse are the views held by the various investigators in the field that we are far from a final solution of the problem".

Many years after Phillips' paper, the "final solution" of the problem is still not at hand. However, modern techniques of biological investigation, such as electron microscopy, microspectrophotometry and electrophysiology, have contributed greatly to unify views on the organization of compound eyes. For example, they have established that the ultimate unit of the compound eye is the retinula cell. Retinula cells have cytoplasmic projections or microvilli whose membrane probably contains photopigment; absorption of light by the photopigment produces an electrical signal (receptor potential) capable of influencing the activity of nerve cells situated centrally.

The eye of the honey bee is among those which have aroused the greatest interest. A detailed description of the structure of the eye of the worker bee is given by Phillips. Many of Phillips' findings were confirmed and extended by Goldsmith (1962), Naka and Eguchi (1962), and very recently by Varela and Porter (1969). The eye of the drone, differing from that of the worker by its larger size, has been described briefly by Naka and Eguchi (1962).

The present work deals in some detail with the ultrastructural organization of the compound eye of the drone. Its aim is to offer a basis for the interpretation of the many electrophysiological data collected in the last years on this eye (Naka and Eguchi, 1962; Autum and von Zwehl, 1964; Baumann, 1968; Fulpius and Baumann, 1969; Shaw, 1969, Hadjilazaro, 1970). Furthermore, this study might be a first step towards a detailed investigation of the metabolism of the retinula cell. Two brief accounts, one dealing with the extracellular space in the drone ommatidium, the other with the number of retinula cells in a single ommatidium, have already been published (Perrelet and Baumann, 1969a, 1969b).

Material and Methods

Heads of the drones were cut by a frontal section passing through both eyes, parallel to the long axis of the ommatidia. The halved heads were fixed for 4 hours in a 4% solution of glutaraldehyde in 0.1 M phosphate buffer pH 7.4 (Sabatini, Bensch and Barrnett, 1963). After a brief rinsing in 0.1 M phosphate buffer, the halved heads were postfixed in 2%

phosphate buffered osmium tetroxide pH 7.4 (Millonig, 1961) for 90 minutes, dehydrated in alcohol and embedded in Epon (Luft, 1961). The embedding was performed in flat capsules (French Nr. MR 1) so that both longitudinal as well as transversal sections of the ommatidia could easily be obtained. Thin sections, placed on parlodion coated grids, were stained with lead citrate (Venable and Coggeshall, 1965) or lead hydroxide (Karnovsky, 1961) and examined in a Philips EM 300 electron microscope fitted with a high resolution or goniometer stage. Thicker sections, stained with 1% toluidine blue in 1% borax, were examined and photographed in a Zeiss photomicroscope.

Histochemical tests were performed in order to ensure the glycogenic nature of the crystalline cone particles (see below). Digestion of glycogen was performed according to the method of Biava (1963), as reported by Phillips and Unakar (1967): after glutaraldehyde fixation, the halved heads were incubated for one hour in saliva at 37° C, then postfixed and embedded as described above. PAS staining (MacManus, 1946), with additional oxydation with 1% $KMnO_4$ before the periodic acid step (Mira-Moser, 1969), was performed on thick Epon sections before and after digestion with saliva.

Finally uranyl-acetate staining in block was sometimes performed according to the technique of Karnovsky (1967) in order to improve the contrast of the plasma membranes.

Observations

1. General Description

Each drone eye is composed of approximately 8,000 photoreceptive units or ommatidia (Chliamovitch, 1969), whose gross morphology is represented in Fig. 1. The ommatidium, formed by 9 visual or retinula cells (Figs. 2, 3), measures approximately 400 μ in length and 20 μ in diameter. Each ommatidium possesses its own dioptric apparatus approximately 100 μ in height, formed by a cuticular corneal facet and a crystalline cone, and is surrounded by a sleeve of elongated pigment cells contiguous with the sleeves of the neighbouring ommatidia. The pigment cells extend from the base of the corneal facet to the basement membrane. The basement membrane, pierced by the retinula cell processes or axons, separates the retina from the optic lobe. The fine structural organization of the dioptric apparatus, of the retinula cells forming the ommatidium, and of the pigment cells will be described successively. In the following description we will make use of the terminology employed by Goldsmith (1964).

2. Fine Structure of the Dioptric Apparatus

The dioptric apparatus of the drone ommatidium is formed by a cuticular corneal facet and a crystalline cone. As in other apposition eyes studied in this respect (Seitz, 1968), the dioptric apparatus of the drone should focus the incident light on the rhabdome. In surface view, and after removal of the corneal hairs (Fig. 4), each facet appears as a convex lens of hexagonal outline. The diameter of the facet is approximately 40 μ and its radius of curvature measures 60 μ. Examined in a scanning electron microscope[1], the surface of the corneal lens appears covered with innumerable bumps approximately 1,000 Å in height (Fig. 4 inset). The total thickness of the corneal facet varies between 35 and 50 μ, the longest ommatidia (see discussion) having the thickest corneal facets. The inner face of the facet is also convex and is separated from the base of the crystalline cone by a narrow cytoplasmic band of corneal pigment cells. In thin sections the

1 Dr. C. Marti, Geigy Ltd., Basle, Switzerland, kindly put a Cambridge Stereoscan scanning electron microscope at our disposition.

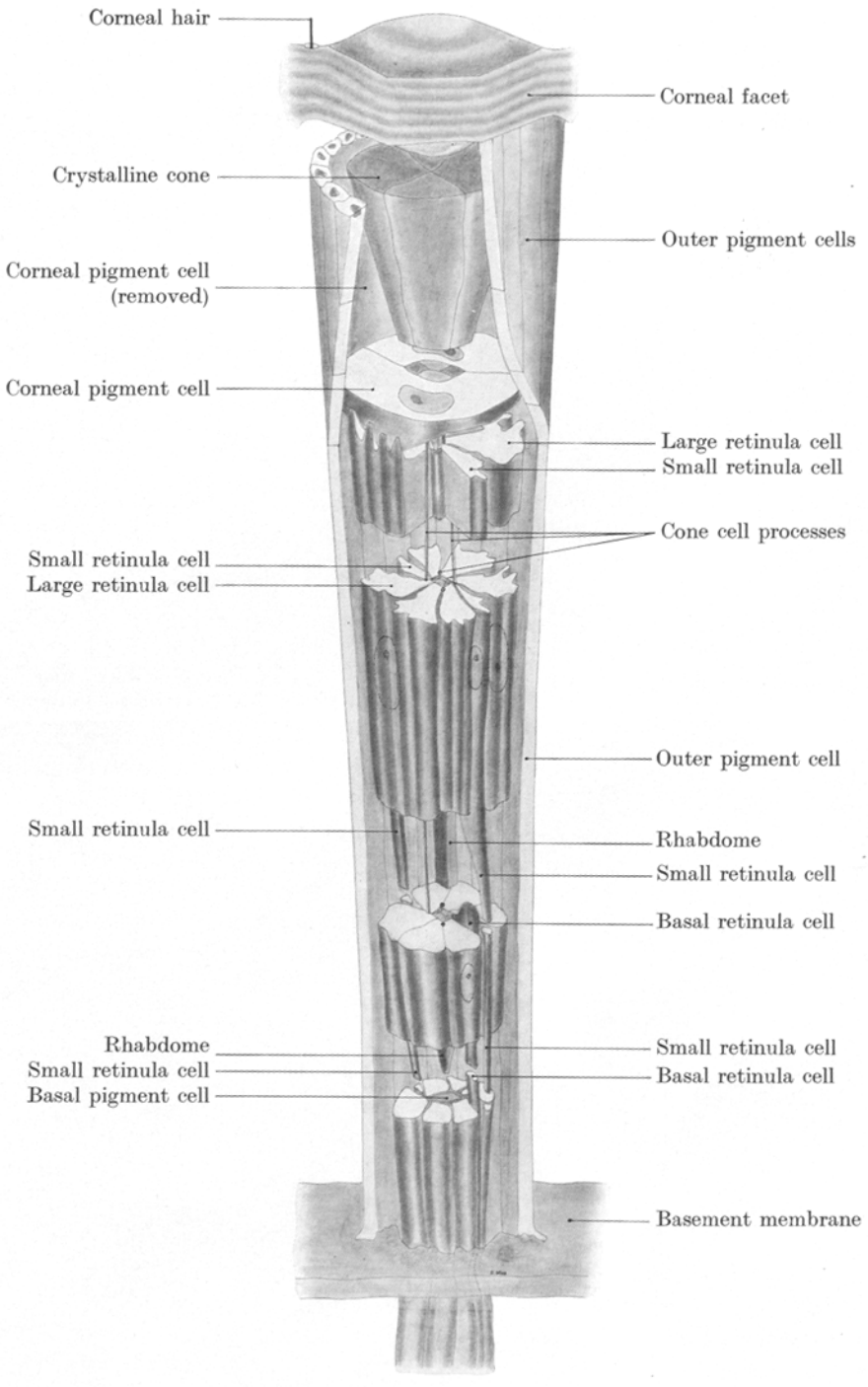
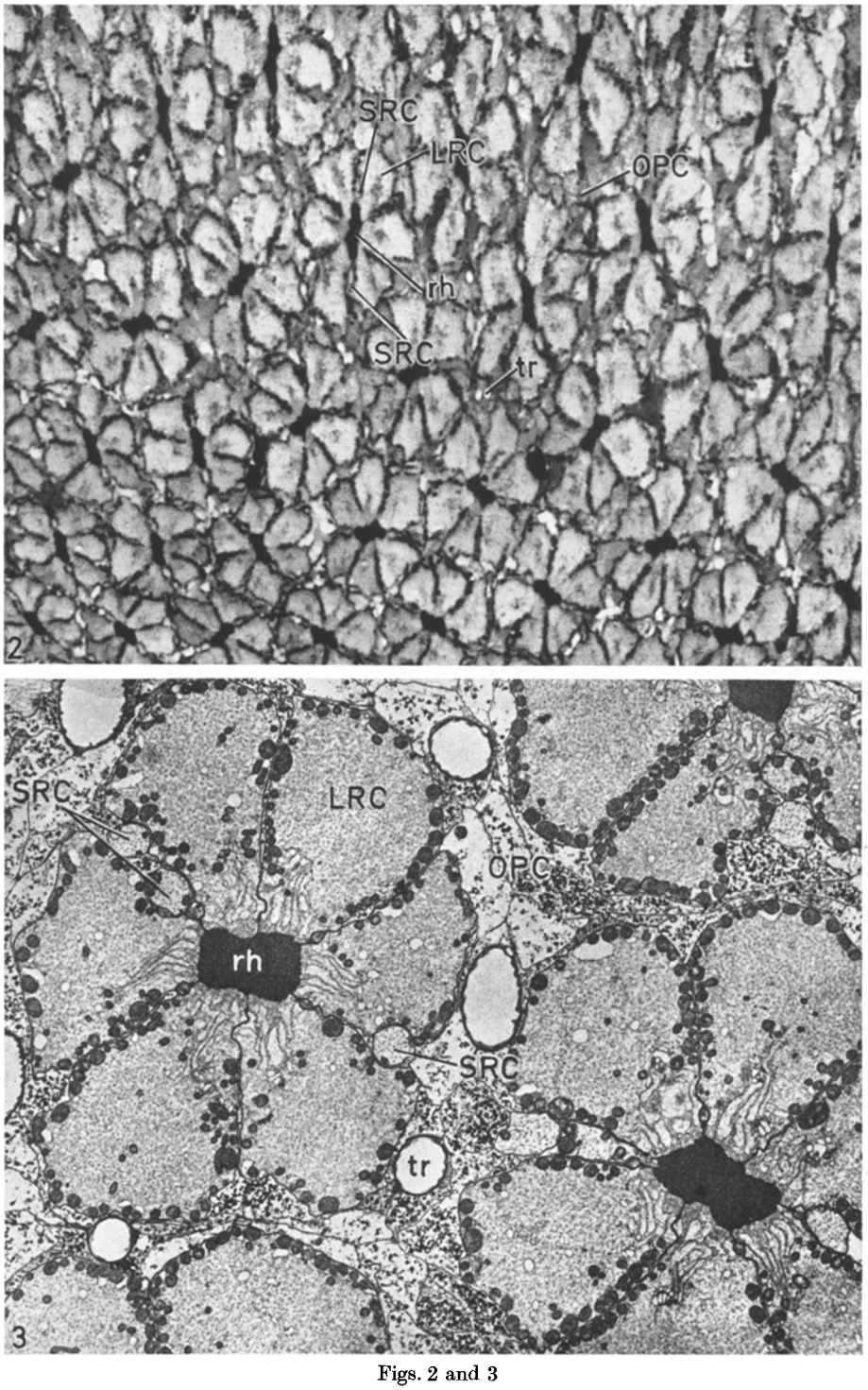


Fig. 1. Semischematic, longitudinal, cutaway view of the honey-bee drone ommatidium



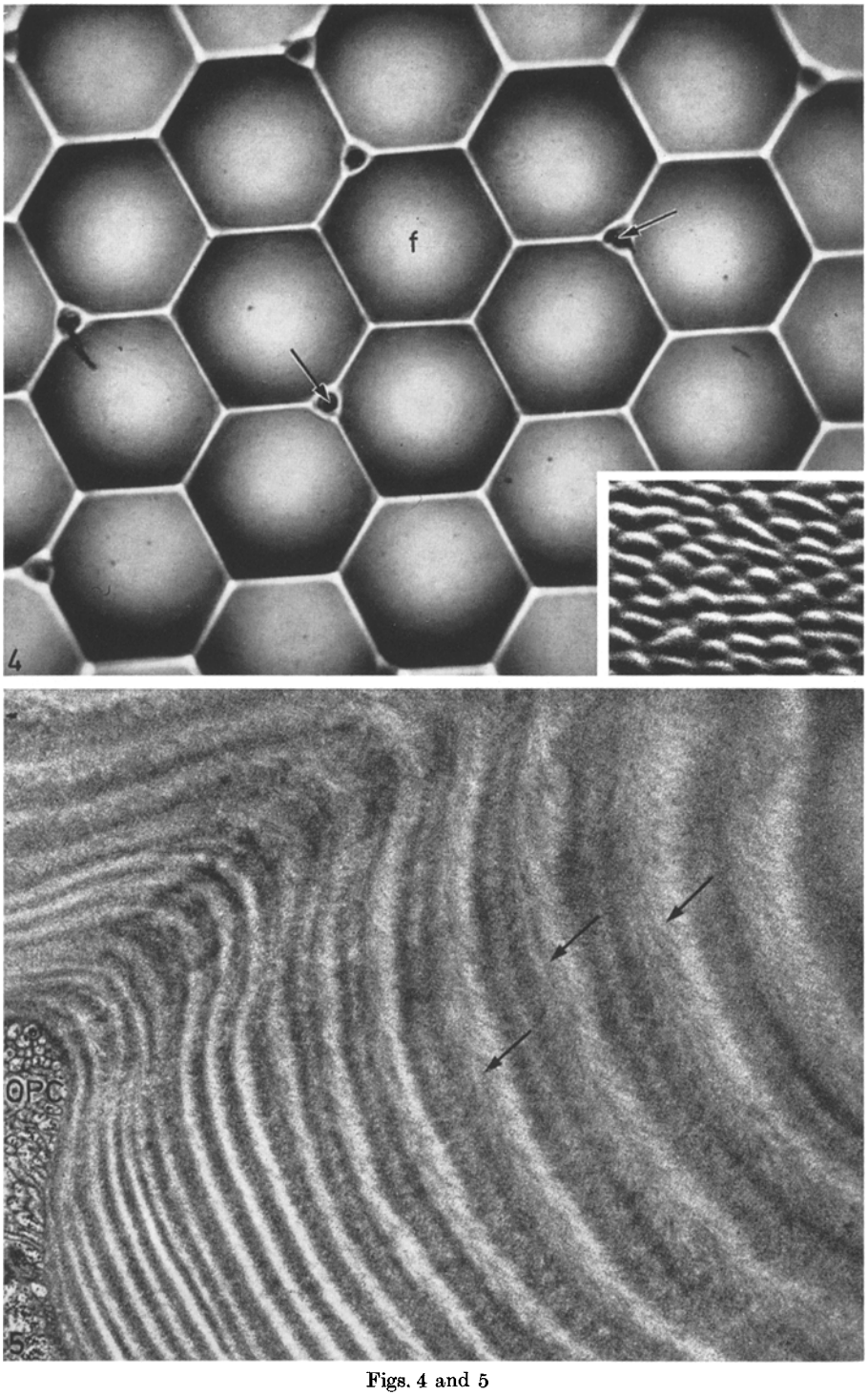
Figs. 2 and 3

corneal stroma has the characteristic appearance of cuticular chitin (Locke, 1964), composed of alternately dense and clear lamellae. The lamellae are formed by curved filaments, easily visible in Fig. 5. Corneal hairs cover the surface of the drone eye. The hairs, 250 μ long, are situated at the intersection of 3 facets (Fig. 4) and, like sensilla (Wigglesworth, 1965), they have been shown to contain a dendrite (Perrelet, 1969). However, neither the cell from which the dendrite arises nor the centripetal axon could be located, in contrast with the results of Sanchez (1920), who described a nerve fiber leaving a bipolar cell at the basis of the hair. In the worker bee, Phillips (1905) did not find evidence for innervation of the hairs, whereas in the pupal stage of *Drosophila* but not in the adult, Waddington and Perry (1960) succeeded in locating nerve fibers in contact with hair cells. Whether eye hair of insects are innervated, and can perform a sensory function, is therefore still undecided.

The crystalline cone is of eucone type (Fig. 6); it measures 25 μ in diameter at its base and its length (approximately 60 μ) varies according to the size of the underlying ommatidium. The tip of the cone is in contact with the rhabdomeric microvilli of the retinula cells, a disposition that is characteristic of apposition eyes (Fig. 7). Each of the four cone cells gives rise to a long cylindrical process inserted between the retinula cells (Fig. 8) and extending down to the basement membrane (Figs. 9, 10). The cytoplasm of the cone cells is devoid of any kind of organelles, except for an elongated nucleus situated towards the outer border and numerous dense particles filling the whole cell body (inset, Fig. 6). Moreover, and particularly near the tip of the cells, the cytoplasm underlying the plasma membrane contains a row of microtubules that enter each cone cell process (inset, Fig. 6). The dense particles filling the cone cell cytoplasm present the characteristic electron microscopical aspect of the so-called beta-particles of glycogen, as described by Drochmans (1962) and Revel (1964). The occurrence of such particles does not seem to be restricted to the eucone cells of the drone since Röhlich and Törö (1965) presented pictures of the eucone of *Daphnia* in which the occurrence of beta-glycogen seems very probable, although these authors interpreted the particles as "ribosome-like". Wolken and Florida (1969) showed micrographs of the eucone of *Copilia* in which similar particles were seen and interpreted as "glycogen-like". In the drone, the glycogenic nature of the dense particles has been studied with the aid of the digesting procedure with saliva. After one hour of digestion, the cytoplasm of the cone cells appears entirely free of dense particles (inset Fig. 25). PAS staining on thick epoxy sections of cones also resulted in a total lack of reactivity once the cones had

Fig. 2. Low power light micrograph of the ommatidia in cross-section (distal level). In each ommatidium one can distinguish the rhabdome (*rh*), six large (*LRC*) and two small (*SRC*) retinula cells. Outer pigment cells (*OPC*) and tracheoles (*tr*) can be seen in between the ommatidia. × 830

Fig. 3. Low power electron micrograph of ommatidia in cross-section (proximal level). This picture shows the rhabdome (*rh*) formed by the six large retinula cells (*LRC*). At this level, the three small cells appear as axons (*SRC*). The ommatidia are separated from each other by several processes of outer pigment cells (*OPC*). Numerous tracheoles (*tr*) are also seen. × 3,060



Figs. 4 and 5

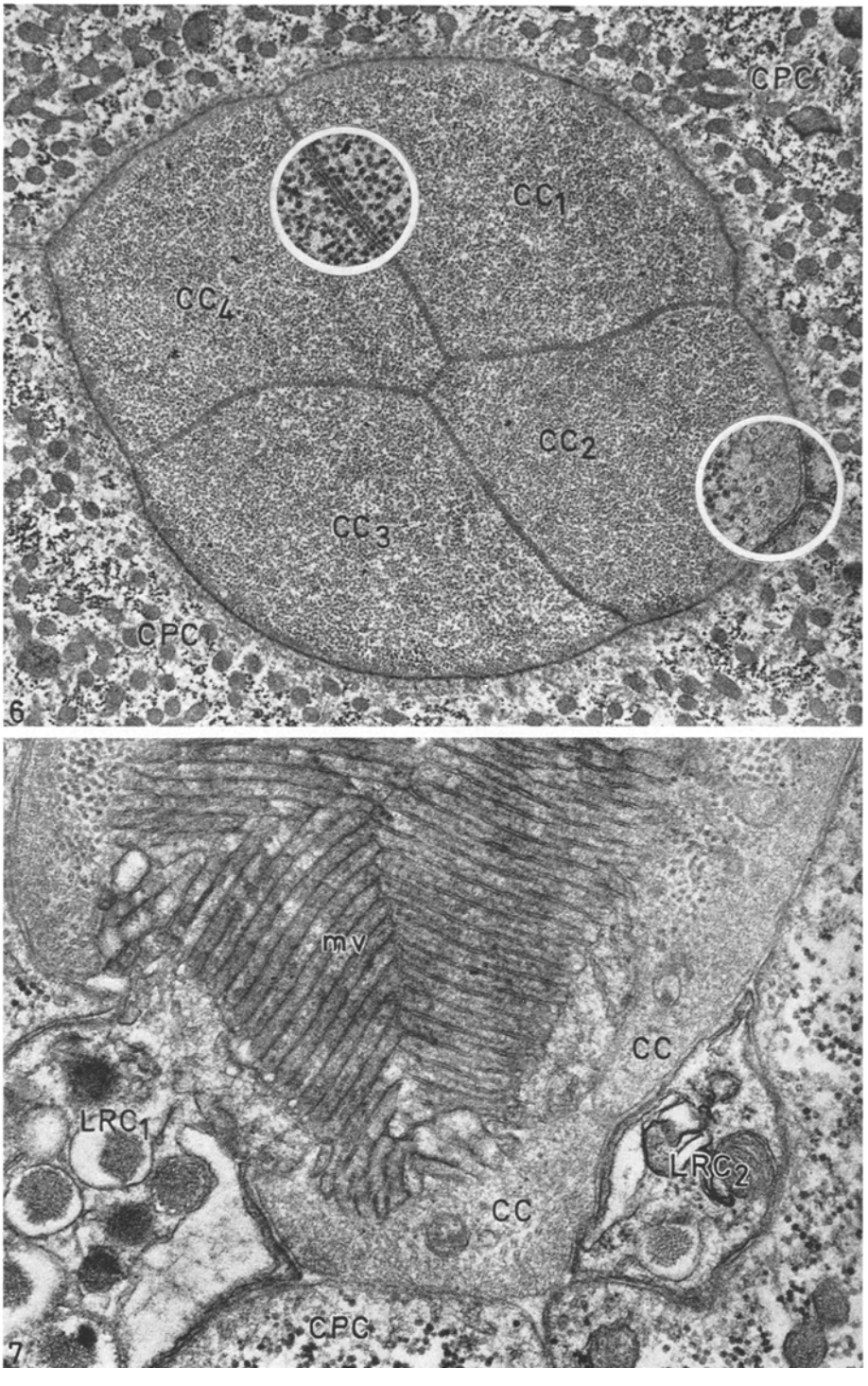
been digested with saliva. On the other hand, the control cones incubated in phosphate buffer without saliva, reacted heavily to PAS staining. The crystalline cone of the worker (Varela and Porter, 1969) is similar in all respects to that of the drone and has been shown to be isotropic (Varela and Wiitanen, as quoted by Varela and Porter). The lack of organelles in cone cells might indicate that glycogen particles cannot be metabolized. Cone glycogen would therefore not have a metabolic function but an optical one, inasmuch as it could contribute to the refractive index.

3. Fine Structure of the Retinula Cells

The ommatidium of the drone is formed by nine retinula cells. As in many other insect species, for example *Locusta* (Horridge, 1965), *Lucilia* (Melamed and Trujillo-Cenóz, 1968), the retinula cells of an ommatidium are not all of equal size. In the drone, six cells are large and three are small (Figs. 9—11, 13). Of the three small retinula cells, one is shorter than the other two and is restricted to the proximal part of the ommatidium. This shorter small cell was described previously and was called the basal retinula cell (Perrelet and Baumann, 1969b). The nine retinula cells are approximately triangular in shape, the top of the triangle being situated in the center of the ommatidium. Here the surface of the retinula cells is covered with numerous, closely packed microvilli. The bulk of microvilli provided by one retinula cell is called a rhabdomere, and together the rhabdomeres of the nine retinula cells form the rhabdome (see below). At the distal end of the ommatidium, the tips of the retinula cells, which lack rhabdomere, are situated at the periphery of the corneal pigment cells (Fig. 1). As the crystalline cone tapers off and disappears, the retinula cells become larger and show well developed rhabdomeres (Figs. 8, 9). At this level the retinula cells are irregular in shape. Their cytoplasm (Fig. 9) is richly provided with organelles, including profiles of rough and smooth endoplasmic reticulum, free ribosomes, Golgi cisternae and mitochondria dispersed at random within the cell. Moreover, numerous pigment granules are present (accessory pigment). The pigment granules, 0.3 to 0.7 μ in diameter, appear as vesicles containing material of variable electron density. Similar pigment granules have been described in the *Limulus* retinula cells by Fahrenbach (1969), who interpreted the dark content as ommochrome. The vesicles with clear fibrillar content could represent early stages in pigment granule formation, as suggested by Shoup (1966) for certain pigment granules in the eye of *Drosophila*. Another cytoplasmic component peculiar to the retinula cells is the system of agranular cisternae situated under the rhabdome (subrhabdomeric cisternae) (Figs. 9, 10). These cisternae do not seem to be in direct

Fig. 4. Surface view of the cornea after removal of the corneal hairs. At certain intersections between 3 hexagonal facets (*f*), the base of the corneal hairs are seen (arrow). × 570. The inset shows the surface substructure as seen in a scanning electron microscope. × 23,000

Fig. 5. Oblique section through a corneal facet. The section reveals the alternately clear and dense lamellae formed by curved filaments (arrow). Processes of outer pigment cells (*OPC*) are seen in contact with the innermost corneal lamella. × 6,400



Figs. 6 and 7

contact either with the rhabdomeric membrane of the retinula cell or with the nonrhabdomeric plasma membrane, but in several instances (Fig. 10) they seem to be continuous with Golgi complexes, or in a few cases, with profiles of granular endoplasmic reticulum. The cisternae undergo swelling during dark adaptation. The cytoplasm is thus organized down to the level of the retinula cell nuclei. These are of ovoid shape and present a conspicuous nucleolus. They all lie in one plane, approximately 100 μ below the distal tip of the retinula cells, except for the basal retinula cell nucleus, which is situated near the basement membrane. The nuclei of the large cells measure approximately 10 μ in length, whereas the small retinula cells have minute nuclei, 4 μ long. Below the level of its nucleus the retinula cell undergoes several changes in its form and in its cytoplasmic organization. The periphery of the cell becomes regular (Fig. 10) and the cytoplasm appears devoid of most of its previous organelles, except for numerous mitochondria, which assume a peripheral disposition in the cell, and the subrhabdomeric cisternae. At this level the three small retinula cells no longer participate in the formation of the rhabdome, and appear as axon-like profiles. Near the basement membrane the rhabdome disappears (Fig. 11) and the large retinula cells are also seen as axons containing numerous microtubules oriented longitudinally (Fig. 12) as well as some mitochondria. The center of the axon bundle is occupied by a dark cell profile containing pigment granules (Fig. 11). This cell will be referred to as the basal pigment cell (see below). In the basement membrane the basal pigment cell disappears and the retinula cell processes form a tight bundle of axons (Fig. 13). Within the bundle, the axons are separated from one another by a thin layer of fibrillar material similar to that forming the basal membrane, and sometimes intermingled with elongated cellular profiles (inset Fig. 13).

As mentioned above, each retinula cell within an ommatidium bears a brush of microvilli called a rhabdomere (Fig. 14). The rhabdome is formed principally by the rhabdomeres of the large retinula cells (Fig. 14). The small retinula cells have only a few microvilli. The peculiar disposition of the six large retinula cells within the ommatidium divides the rhabdome into four parts, determined by the orientation of the microvilli (Fig. 14). As illustrated in Fig. 17, the rhabdome varies in shape and size according to the level of the ommatidium. By adding the surfaces obtained at all levels, it may be concluded that the rhabdome resembles a parellelepiped, widening slightly towards its middle. The rhabdomeric microvilli are cylinders averaging 0.5 μ in length and 0.08 μ in diameter (Fig. 15). They contain an irregular dense core and are limited by a unit membrane, 90 Å

Fig. 6. Cross-section of the crystalline cone revealing the four cone cells (CC_1-CC_4) filled with glycogen particles (inset). The periphery of the cells contains numerous microtubules (inset). Parts of the corneal pigment cells (*CPC*) surround the cone. × 12,900; insets × 33,700

Fig. 7. Cross-section of the transitional zone between the tip of the crystalline cone and the rhabdomeric microvilli. Due to the peculiar geometry of this region (see Fig. 1 and next figure), the microvilli (*mv*) on the left hand of the figure appear continuous with the retinula cell (LRC_1), whereas those on the right hand are separated from the retinula cell (LRC_2) by a band of cone cells (*CC*). Both retinula cells are surrounded by corneal pigment cells (*CPC*). × 31,600

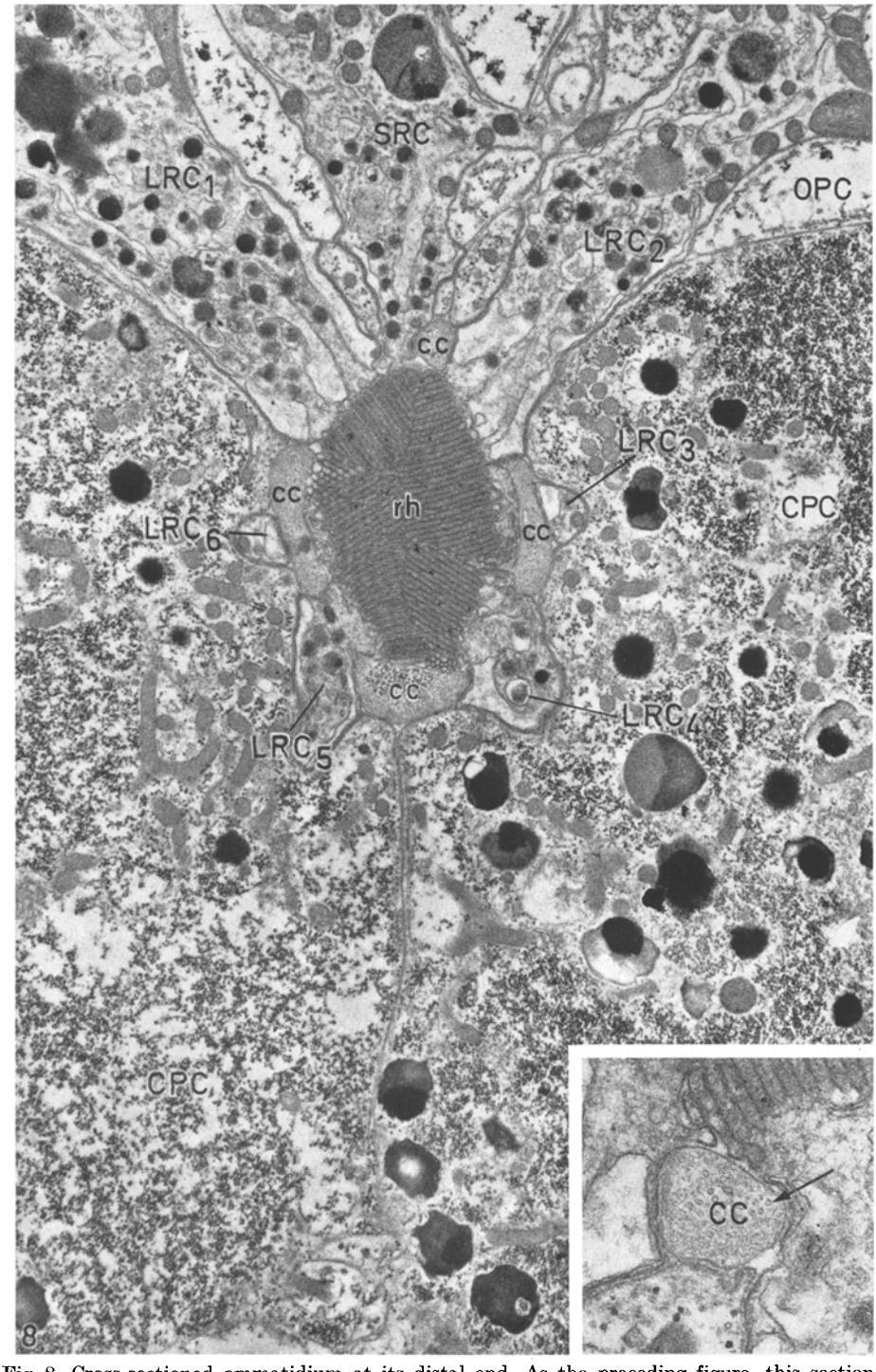


Fig. 8. Cross-sectioned ommatidium at its distal end. As the preceding figure, this section reveals the unequal peripheral extension of the retinula cells. Three of these (LRC_1, SRC, LRC_2) are large and surrounded by outer pigment cells (OPC), whereas the others (LRC_3-LRC_6) are small and surrounded by corneal pigment cells (CPC). One of the cone cell processes (cc) contains glycogen particles. Glycogen deposits are conspicuous in the corneal pigment cells. The difference between the pigment granules of the retinula cells and those of the corneal pigment cells is apparent. $\times$ 11,900. The inset displays the fine structure of a cone cell process with its conspicuous microtubules (arrow). $\times$ 27,200

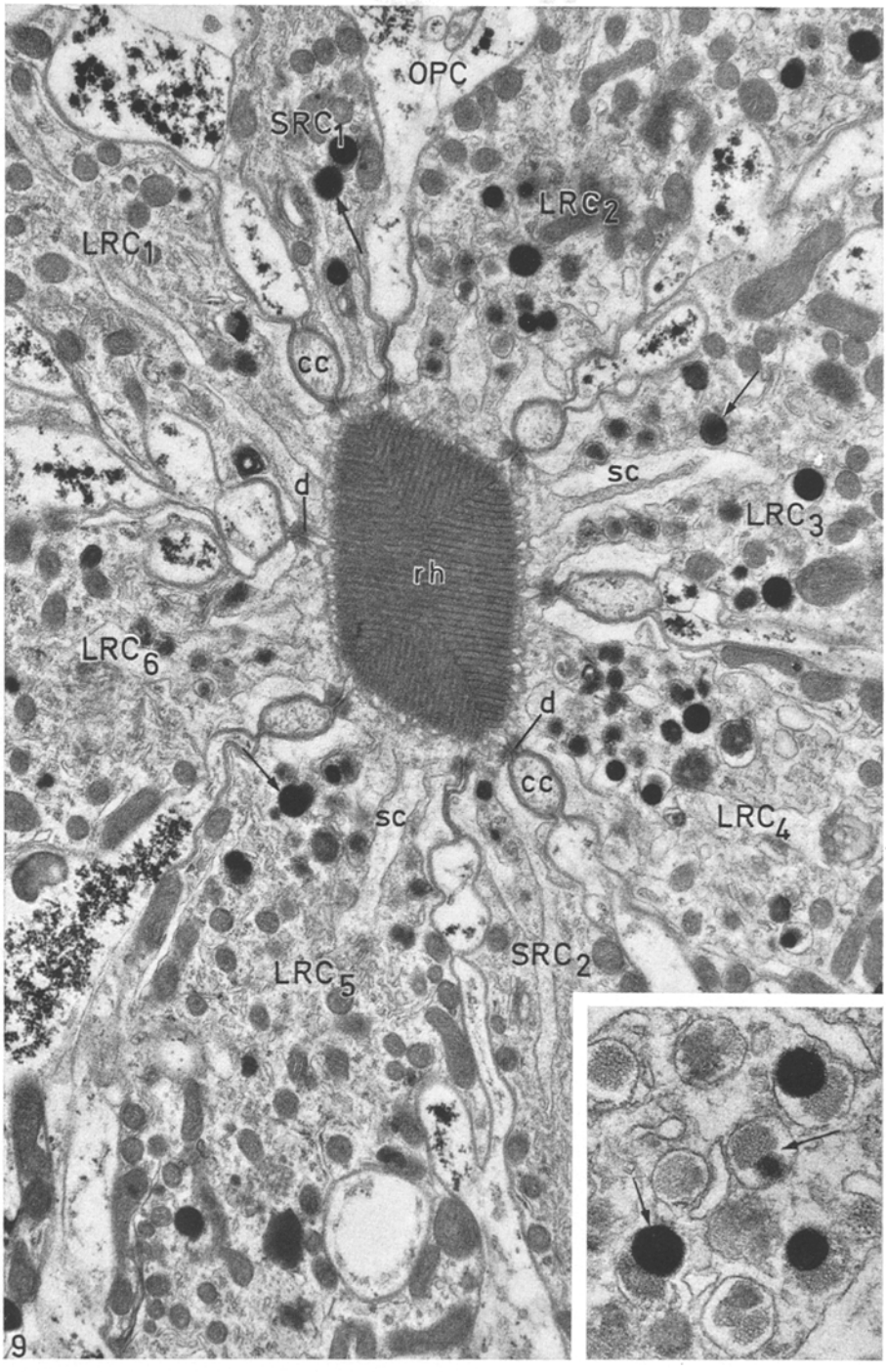


Fig. 9. Cross-section of an ommatidium above the level of the retinula cell nuclei. Around the rhabdome (*rh*) one can distinguish the 6 large (LRC_1—LRC_6) and the 2 small (SRC_1—SRC_2) retinula cells. All retinula cells contain numerous accessory pigment granules (arrow), various organelles and subrhabdomeric cisternae (*sc*). The four cone cell processes (*cc*) can be seen beyond the zonula adhaerentes (*d*). Outer pigment cells (*OPC*) are wedged in between the retinula cells. × 15,500. Accessory pigment granules of the retinula cells are pictured in the inset (arrows). × 30,000

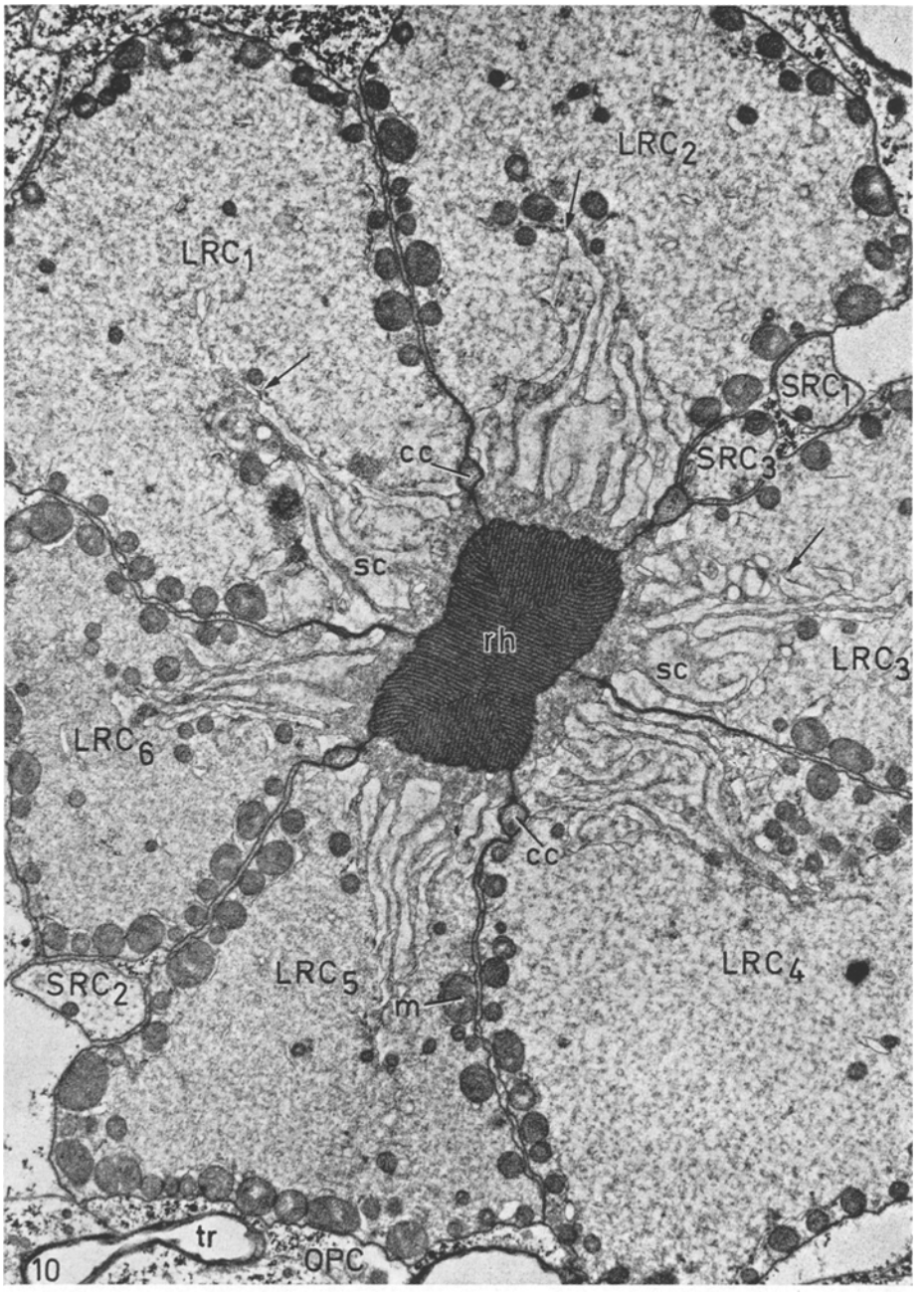


Fig. 10. Cross-section of an ommatidium below the level of the retinula cell nuclei. The six large retinula cells (LRC_1—LRC_6) forming the rhabdome (*rh*) have a regular shape. The three small cells (SRC_1—SRC_3) appear as axon-like profiles. Retinula cell cytoplasm still contains subrhabdomeric cisternae (*sc*) and numerous mitochondria (*m*). Arrows point to the connection between cisternae and Golgi apparatus or rough endoplasmic reticulum. Tracheoles (*tr*) are seen between the outer pigment cells (*OPC*). × 8,200

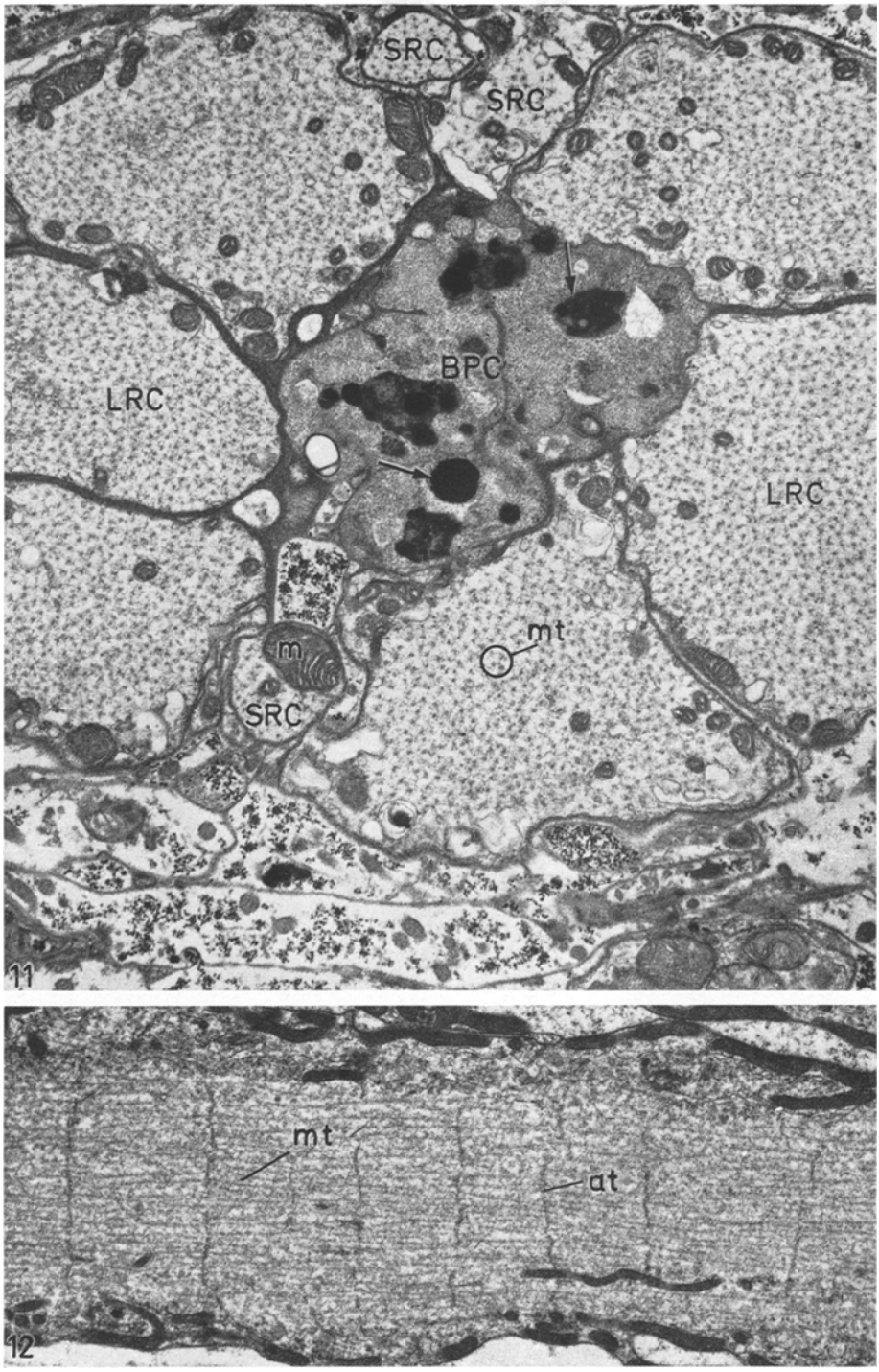


Fig. 11. Cross-sectioned ommatidium near the basement membrane. At this level, the center of the ommatidium is occupied by processes of the basal pigment cell (*BPC*) rich in pigment granules (arrow). All retinula cells (*LRC, SRC*) appear as axons containing microtubules (*mt*) and mitochondria. × 10,800

Fig. 12. Longitudinal section of a retinula cell axon showing the longitudinally oriented microtubules (*mt*) and the transverse agranular tubules (*at*). × 7,800

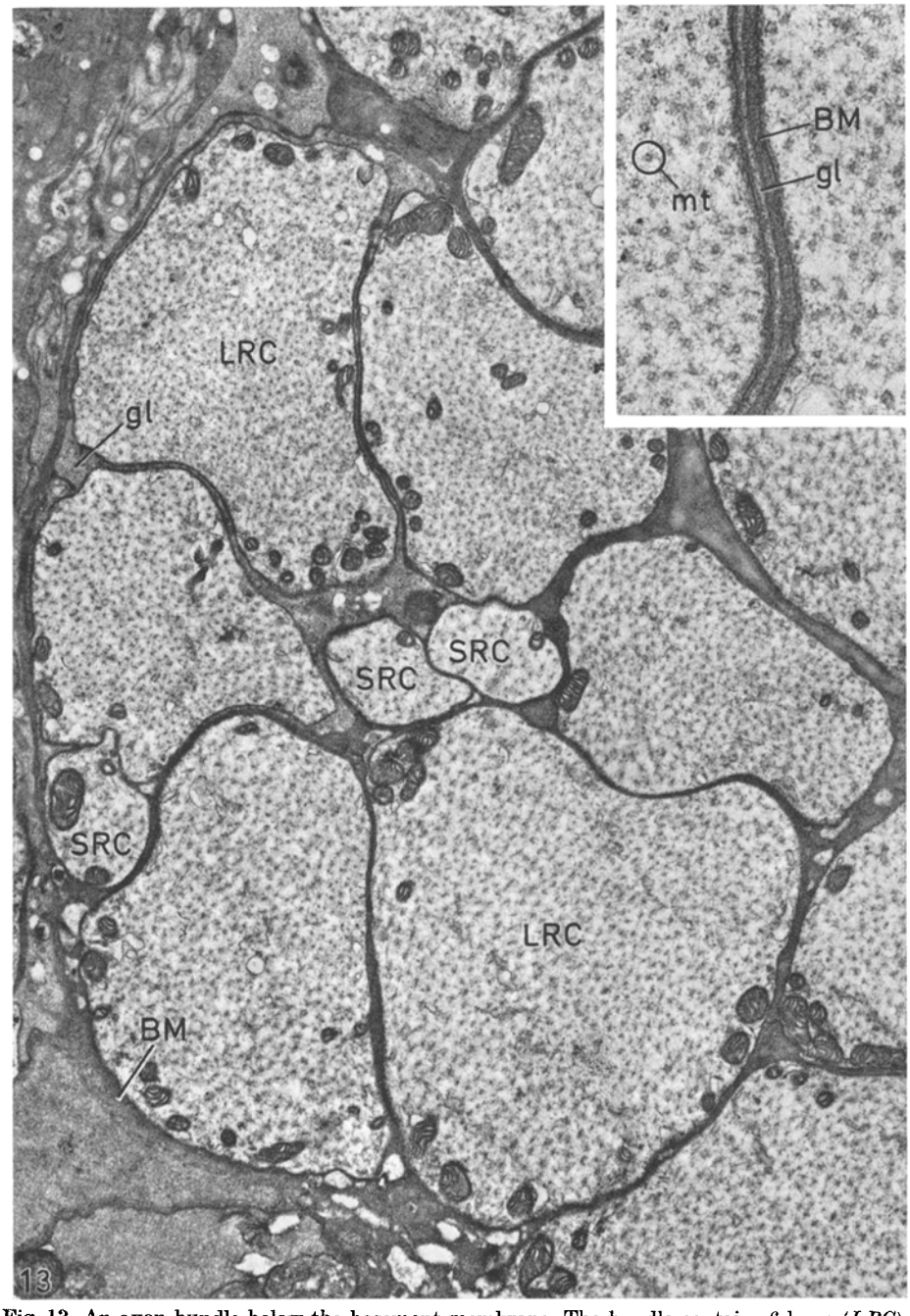


Fig. 13. An axon bundle below the basement membrane. The bundle contains 6 large (*LRC*) and 3 small (*SRC*) axons. The axons are separated by basement-membrane-like material (*BM*) and in some cases by slender cell profiles of glial origin (*gl*). × 8,200. The inset displays glial cell profile and basement-membrane-like material at high magnification. Microtubules (*mt*) within the axons are conspicuous. × 32,100

thick, which is finely beaded in appearance (Fig. 16). This aspect of the membrane of the microvilli was also described in other photoreceptor membranes, in both invertebrates and vertebrates (Fernandez-Morán, 1962; Blasie *et al.*, 1965; Nilsson, 1967; White, 1968; Fahrenbach, 1969) and has been interpreted as possible evidence for the presence of photopigment molecules (Fahrenbach, 1969). The rhabdomeric microvilli are tightly packed and the outer leaflets of the limiting membrane touch each other, forming tight junctions (Fig. 16). The extracellular space in the rhabdome is therefore confined to the narrow triangular spaces between adjacent microvilli. These spaces have been shown to contain an extracellular material of appreciable electron density (Perrelet and Baumann, 1969a). A calculation based on the average measurements of the microvilli indicates that a rhabdome contains approximately $6 \cdot 10^5$ microvilli. The total surface of the microvilli amounts to $1.18 \cdot 10^5\ \mu^2$, whereas the surface of the non-rhabdomeric area of the six large retinula cells represents $0.7 \cdot 10^5\ \mu^2$ only.

4. Junctional Specializations and Extracellular Space within the Ommatidium

The search for junctional specializations between the different cell types of the retina presents particular functional interest because of the role of certain junctions in restricting extracellular fluid movement as well as in electrical coupling between cells. In the drone retina this search was rendered difficult by the fact that the usual fixation and staining procedures do not suffice to bring out the "unit" structure of the cell membranes. However, in suitable areas of tissue stained in block with uranyl acetate, the unit membrane structure was clearly visible. Four basic types of junctional specialization have been found: tight junctions (Farquhar and Palade, 1963), adhering junctions (Farquhar and Palade, 1963), gap junctions (Revel and Karnovsky, 1967) and septate desmosomes (Wood, 1959). The tight junctions between the rhabdomeric microvilli have already been mentioned (Fig. 16). Beyond the rhabdome, the retinula cells are joined by short adhering junctions (zonulae adhaerentes), running the whole length of the ommatidium (Figs. 8—10). These junctions disappear when the retinula cells become axons (Fig. 11). The outer pigment cells unite for stretches of so-called gap junctions as illustrated in Fig. 21a and b; between this latter cell type and the corneal pigment cells, junctional specializations resembling septate desmosomes (Fig. 22) were detected by uranyl acetate deposit. In the distal part of the ommatidium, long processes of outer pigment cells are deeply wedged in between the retinula cells. These two cell types were always shown to be separated by an extracellular space measuring 150—200 Å in width (Fig. 20). In the proximal part of the ommatidium, however, the outer pigment cells do not protrude as deeply in between the retinula cells (Fig. 10). In the areas not penetrated by pigment cells, the membranes of the retinula cells come close to one another but goniometric analysis of such areas (Figs. 18, 19a, b) still reveals the presence of a 100 Å gap between the retinula cells. These results, as well as the previously demonstrated penetration of diffusion tracers between the rhabdomeric microvilli (Perrelet and Baumann, 1969a), favour the hypothesis that the retinula cell membrane bathes in the extracellular fluid through open channels between cells.

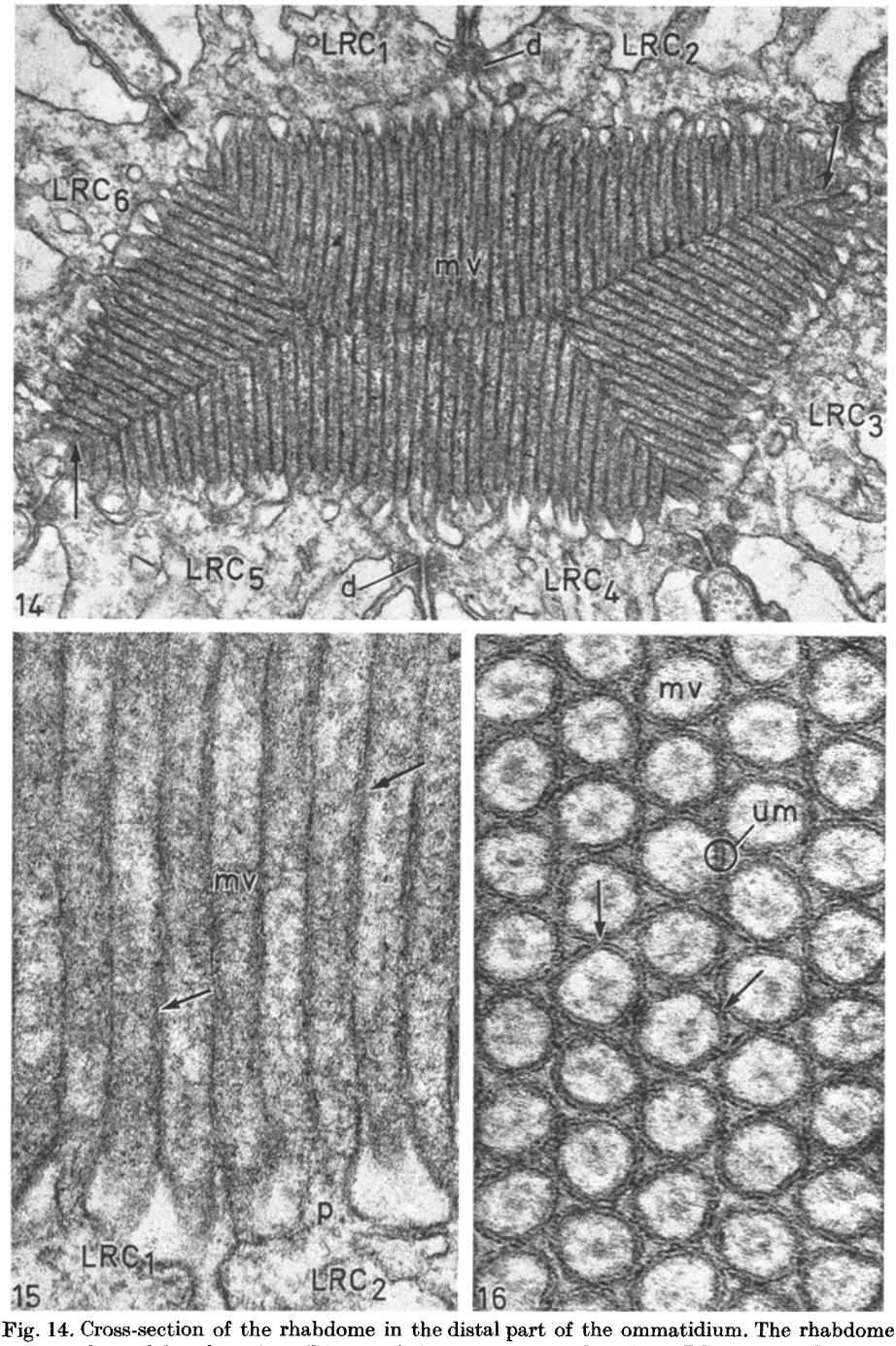


Fig. 14. Cross-section of the rhabdome in the distal part of the ommatidium. The rhabdome appears formed by the microvilli (*mv*) of the 6 large retinula cells (LRC_1—LRC_6). The two small cells present at this level have both few and short microvilli (arrow). The division of the rhabdome in four parts as well as adhering junctions (*d*) between retinula cells, are clearly visible. × 22,200

5. *Fine Structure of the Pigment Cells*

Three types of pigment cells are found in the drone retina: the corneal, the outer, and the basal pigment cells. In these cells the pigment is called accessory or screening, indicating that it has no photosensitive function. Chemically, the accessory pigments are pterins and ommochromes (Butenandt *et al*, 1958); in the pigment cells they usually appear bound to cytoplasmic granules (Goldsmith, 1964).

The corneal pigment cells are two large cells which surround the crystalline cone (Fig. 1). The distal part of the corneal pigment cells is narrow (Fig. 23), whereas the proximal part, at the tip of the crystalline cone, appears wide and contains the nucleus (Fig. 24). The cytoplasm is rich in dense particles which have the same morphological characteristics as those of the crystalline cone and whose glycogenic nature is indicated by the fact that they are also digested by incubation in saliva (inset Fig. 25). A row of microtubules is present on the inner side of the cells, facing those of the crystalline cone (Fig. 25). Pigment granules are concentrated in the proximal part of the cell and appear in two principal forms. Some granules (Fig. 26) are small, roundish and of homogenous electron density, resembling those already described in the distal cytoplasm of the retinula cells (Fig. 9). Other granules occurring more frequently, are large and irregular (Fig. 27). The matrix of these granules is of variable electron density and viewed at high magnification it appears fibrillar (Fig. 28). In addition to the pigment granules, the cytoplasm of the corneal pigment cells contains structures that resemble primary lysosomes containing glycogen particles.

The outer pigment cells encompass the entire ommatidium from the corneal facet to the basement membrane. There are 27 to 31 outer pigment cells around each ommatidium. These cells, roughly cubical in their distal part (Fig. 23) containing the nucleus, taper off progressively (Figs. 10, 29) in the basal region of the retina. They adhere to the basement membrane and to the inner surface of the cornea by small digitations (Figs. 5, 29 insets). The cytoplasm of the outer pigment cells also contains glycogen particles, as well as numerous microtubules (Fig. 29). With the fixative used for this study, the outer pigment cells were usually less well preserved than the other cell types of the ommatidium. This difference remains unexplained as yet. Pigment granules abound in the proximal and distal extremities of the cells (Figs. 29, 30), and are scarce in the middle part. Both types of pigment granules described in the corneal pigment cells are present in the outer pigment cells. The first type predominates in distal extremities, the second type in the proximal part of the cells, near the

Fig. 15. High magnification of the rhabdomeric microvilli in longitudinal section. The microvilli (*mv*) are attached to the retinula cell surface (LRC_1-LRC_2) by a narrow pedicule (*p*). This picture shows the close contact between adjacent microvilli (arrow). × 83,500

Fig. 16. High magnification of rhabdomeric microvilli in transverse section. The unit structure of the plasma membrane (*um*) can be seen clearly. Close contact points between microvilli (tight junctions) are indicated by arrows; uranyl-acetate staining in block. × 145,800

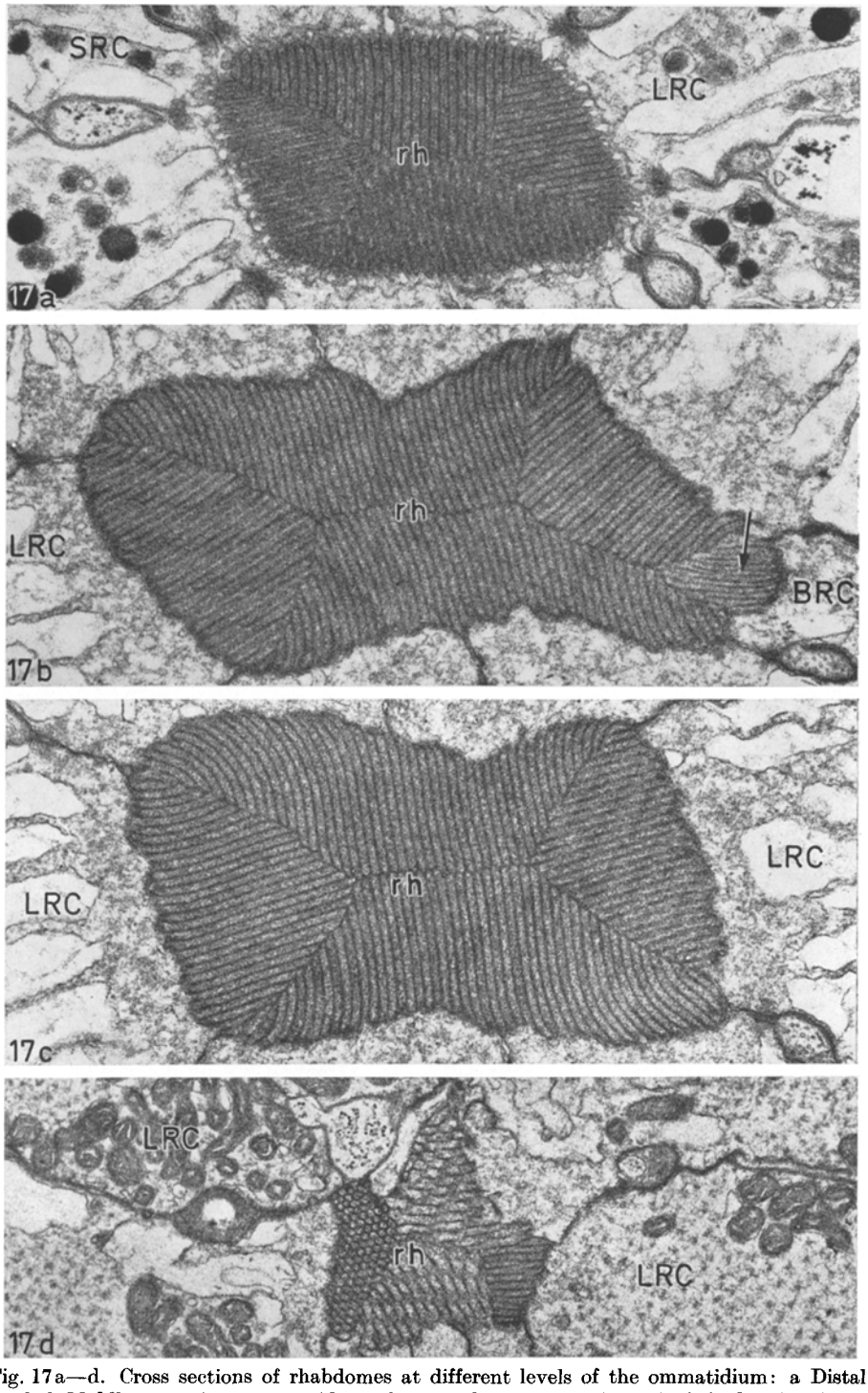


Fig. 17a—d. Cross sections of rhabdomes at different levels of the ommatidium: a Distal level; b Middle part of the ommatidium showing the microvilli (arrow) of the basal retinula cell (*BRC*); c Basal part of the ommatidium; d Proximal end of the rhabdome (cf. Fig. 1). Magnification is the same for the four figures. × 18,500

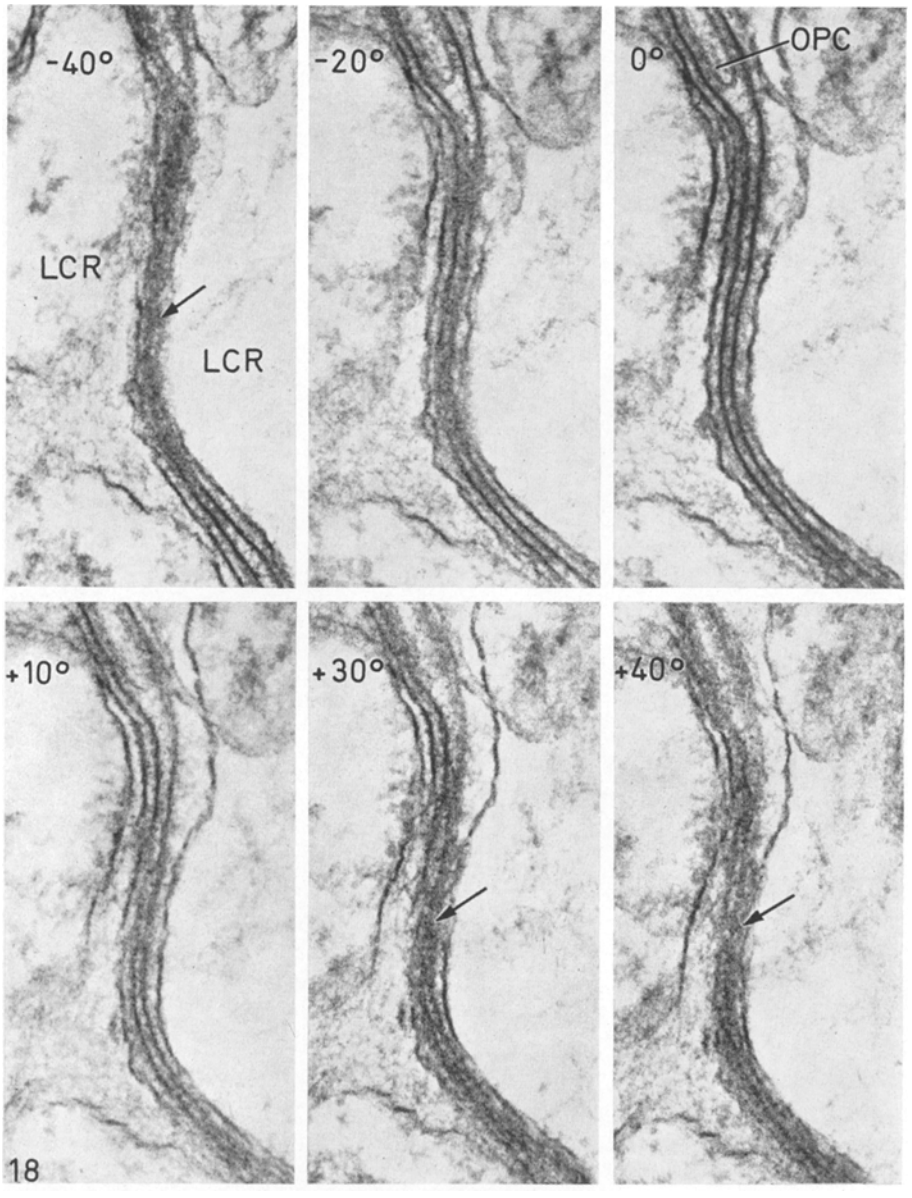


Fig. 18. Goniometric analysis of a junction between two retinula cells (*LCR*). At extreme tilt angles, simulating tangential sections (−40°, +30° and +40°), the retinula cell membranes appear fused (arrow). An extracellular gap is however clearly visible at 0 tilt angle, representing a plane of section perpendicular to the retinula cell membrane. Cf. next figure showing densitometric tracing of this picture. × 67,000

basement membrane. The relationships between retinula cells and outer pigment cells, as well as between outer pigment cells, have been described with regards to the junctional specializations in the ommatidium. Tracheoles, whose cell

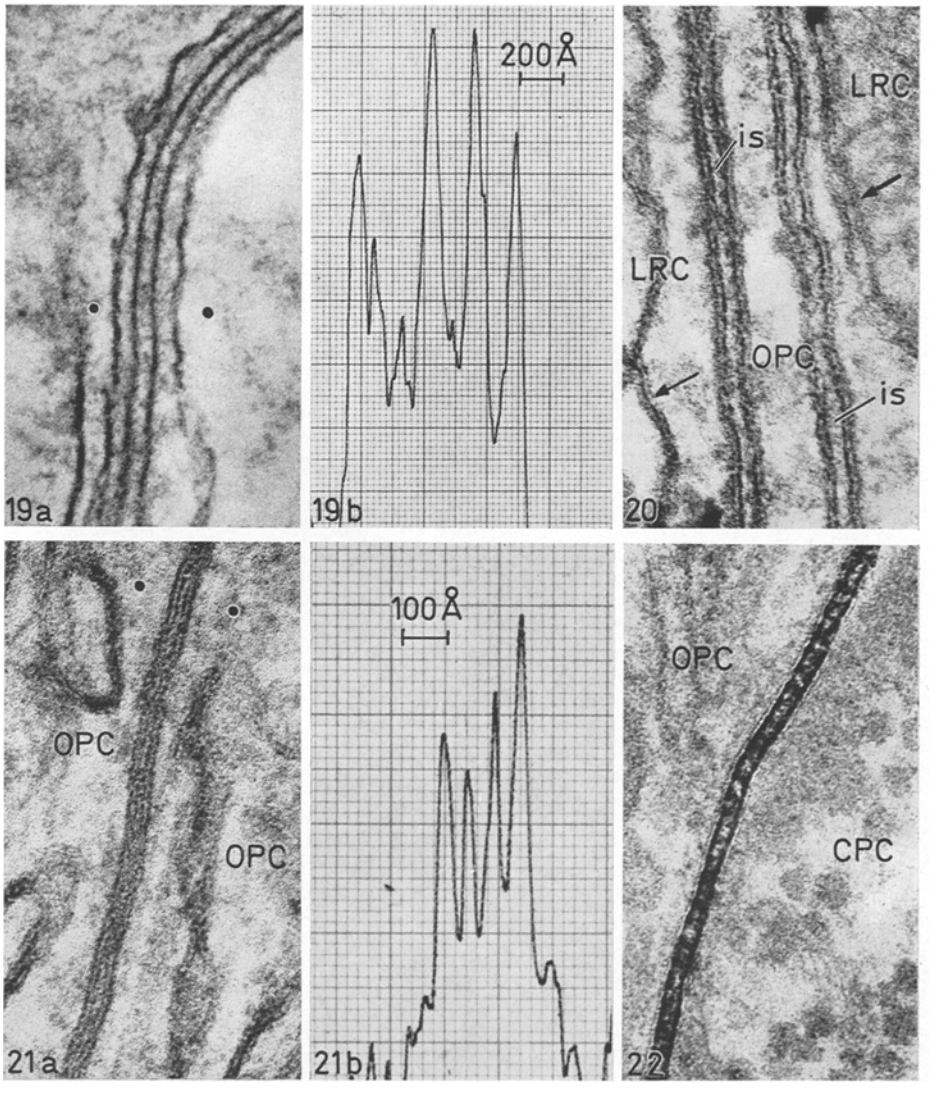


Fig. 19a and b. Same zone as in Fig. 18 (0°). × 102,000. b The densitometric tracing performed across the two membranes, from one dark dot to the other (Fig. 19a) reveals a gap approximately 100 Å wide between the two retinula cells. The two large peaks of the densitometric trace are considered as the centers of the retinula cell membranes. A maximal thickness of 100 Å for the unit membrane was assumed in evaluating the gap. × 272,000

Fig. 20. Intercellular spaces (*is*) between retinula cells (*LRC*) and an outer pigment cell (*OPC*) process. Arrows point to intracytoplasmic membranes. Uranyl-acetate staining in block reveals the unit membrane of both retinula and pigment cells. × 152,000

Fig. 21a and b. Gap junction between two outer pigment cells (*OPC*). × 152,000. b The densitometric tracing performed across the gap junction, from one dark dot to the other (Fig. 21a), indicates the presence of a small extracellular gap 30 Å wide. × 600,000

Fig. 22. Intercellular uranyl-acetate deposit revealing the presence of a septate desmosome between outer (*OPC*) and corneal (*CPC*) pigment cells. × 133,000

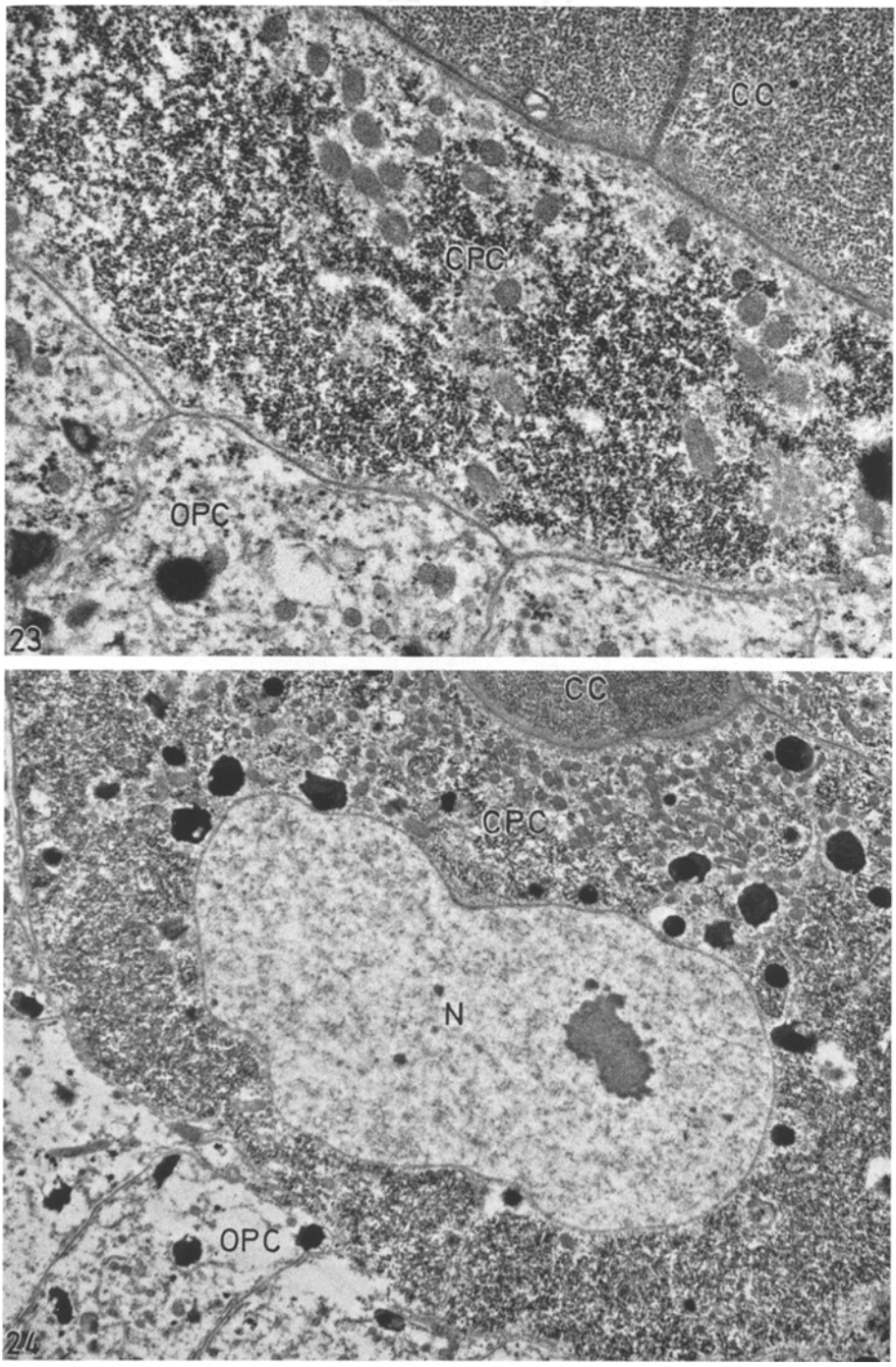
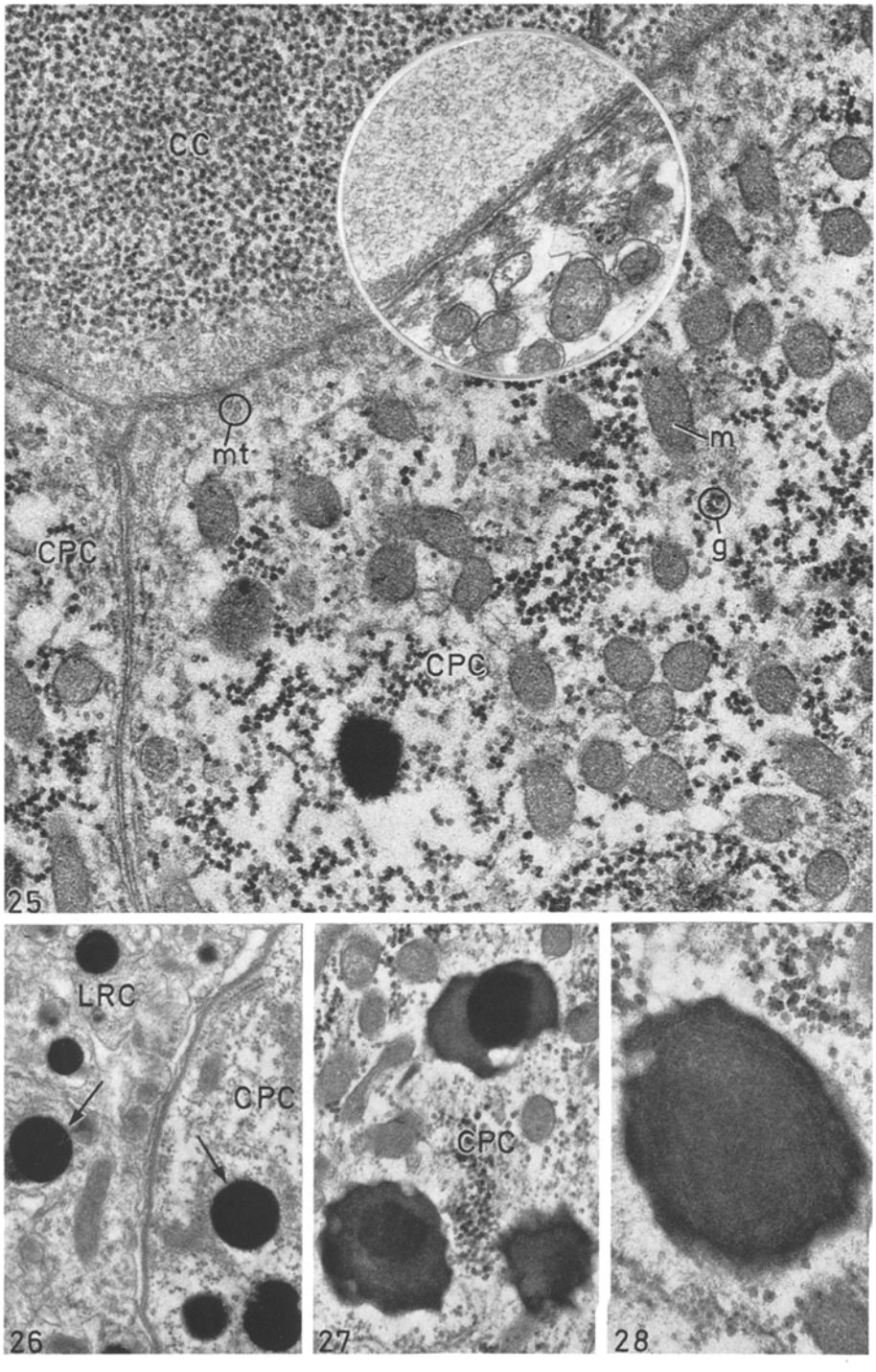


Fig. 23. Cross-section of a corneal pigment cell at its distal level. The cell (*CPC*) which surrounds the crystalline cone (*CC*) is narrow and contains a large amount of glycogen particles but no pigment granules. Outer pigment cells (*OPC*) are visible at the periphery of the corneal pigment cell. × 15,900

Fig. 24. Cross-section of a corneal pigment cell near the tip of the crystalline cone (*CC*). At this level, the cell (*CPC*) is large and contains a conspicuous nucleus (*N*), glycogen particles and numerous pigment granules. Outer pigment cells (*OPC*) surround the corneal pigment cell. × 9,500



Figs. 25—28

bodies are situated below the basement membrane, wander in between the outer pigment cells (Figs. 10, 30). Numerous in the proximal part of the retina, the tracheoles become rare in distal regions where they ultimately disappear. In most instances the tracheal tubes are separated from the membrane of the retinula cell by a narrow band of outer pigment cell cytoplasm.

The base of the ommatidium is occupied by dark cell profiles containing numerous large and dense pigment granules (Figs. 11, 31), some of which appear trapped in lysosome-like structures[2] (inset Fig. 31). These dark cells, referred to as the basal pigment cells, usually lack glycogen deposits; their central position in the ommatidium makes them easily distinguishable from the narrow, elongated outer pigment cell processes adhering to the basement membrane. Similar cells have been described by Varela and Porter (1969) in the worker bee retina, as well as by Fahrenbach (1969) in the *Limulus* ommatidium. Their presumable function is that of catching the light that remains unabsorbed by the rhabdome (Varela and Porter, 1969).

In addition to the three types of pigment cells described above, extremely narrow cellular profiles can be seen wrapping the axon bundle at the base of the retina (Fig. 13). Similar cell profiles in the worker bee retina have been considered by Varela and Porter (1969) to be processes of glial cells situated below the basement membrane.

Discussion

The compound eye of the drone is a typical apposition eye with closed-type rhabdomes. The ommatidium is formed by 9 retinula cells and is associated with 3 types of pigment cells. The number of retinula cells found in the course of this study differs both from that reported by Naka and Eguchi (1962) in a paper on the drone ommatidium, as well as from the number of retinula cells noted in the worker ommatidium (Goldsmith, 1962; Varela and Porter, 1969). All these authors reported 8 retinula cells forming either the drone or the worker bee ommatidia. The findings of Naka and Eguchi like those of Goldsmith, can

Fig. 25. High magnification of crystalline cone cells and corneal pigment cells. This figure shows the cytoplasmic organization of the corneal pigment cell (*CPC*): microtubules (*mt*) facing those of the cone cells, numerous mitochondria *(m)* and beta-particles of glycogen (*g*). × 33,700. The inset represents part of cone cell and corneal pigment cell after digestion with saliva. All glycogen particles have disappeared, leaving only the row of microtubules and a light fibrillar network. × 33,700

Fig. 26. Accessory pigment granules (arrow) in the distal tip of a retinula cell (*LRC*) and corneal pigment cell (*CPC*). × 11,700

Fig. 27. Accessory pigment granules in a corneal pigment cell (*CPC*). × 25,900

Fig. 28. High magnification of an accessory pigment droplet with fibrillar structure. This droplet belongs to a corneal pigment cell. × 48,600

2 Secretion granules trapped in lysosome-like structures were described by Smith and Farquhar (1966) and by Orci *et al.* (1968) in endocrine cells of the rat. Called granulolysis by Orci *et al.*, this phenomenon was interpreted as a cellular mechanism for disposing of excess secretory product in certain metabolic conditions. The possible role of "granulolysis" in basal pigment cells is not known so far.

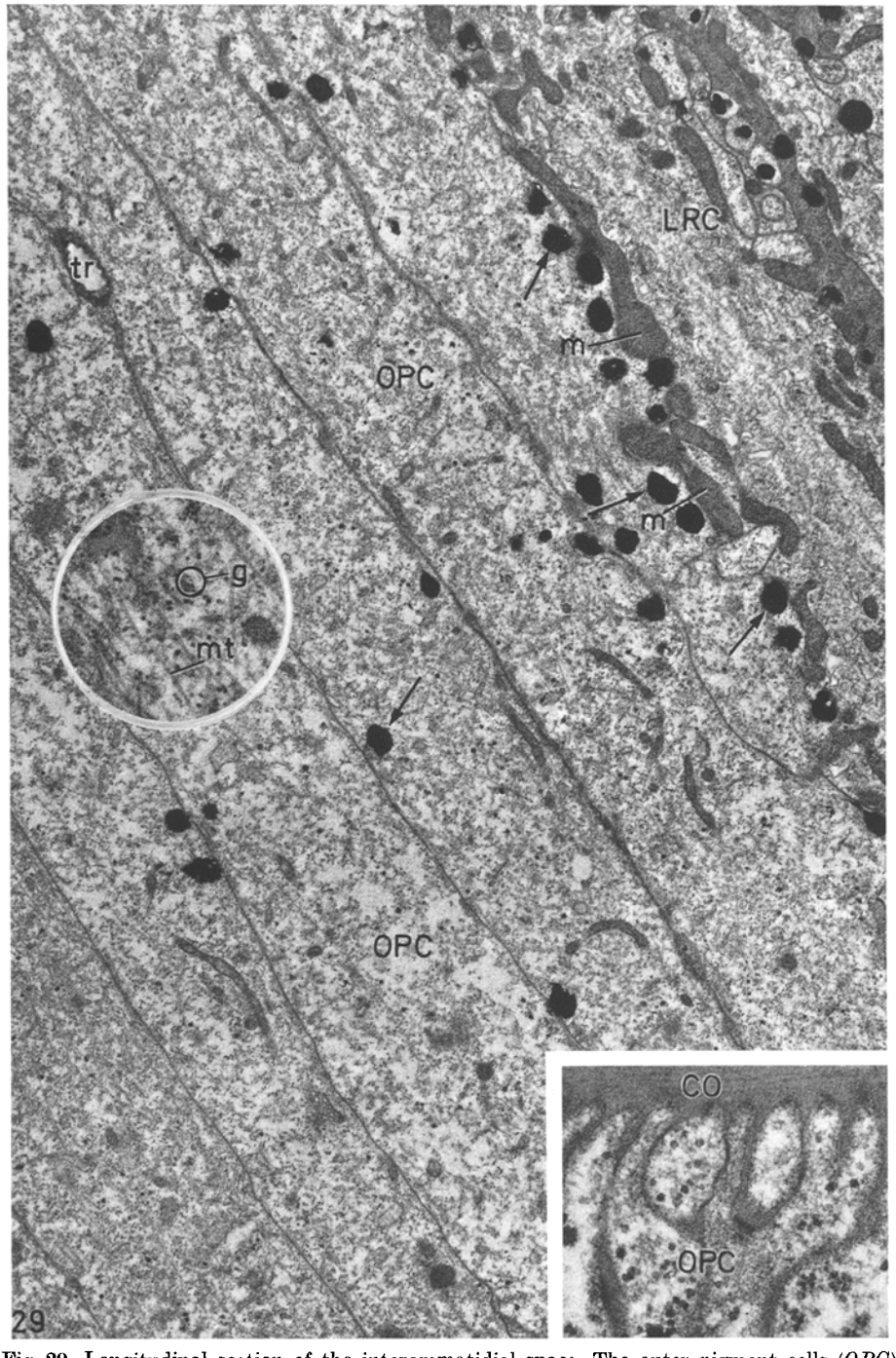


Fig. 29. Longitudinal section of the interommatidial space. The outer pigment cells (*OPC*) appear as several elongated profiles. In the upper right hand corner, a retinula cell (*LRC*) is visible. Its cytoplasm differ from that of the pigment cells by large and numerous mitochondria (*m*). Pigment cell cytoplasm contains microtubules (inset) beta-glycogen particles (inset) and accessory pigment granules which are particularly numerous close to the retinula cell membrane (arrow). $\times$ 7,600 (inset $\times$ 22,700). The attachment of the distal tip of the outer pigment cells (*OPC*) on the inner corneal surface (*CO*) is pictured in the square inset. $\times$ 33,600

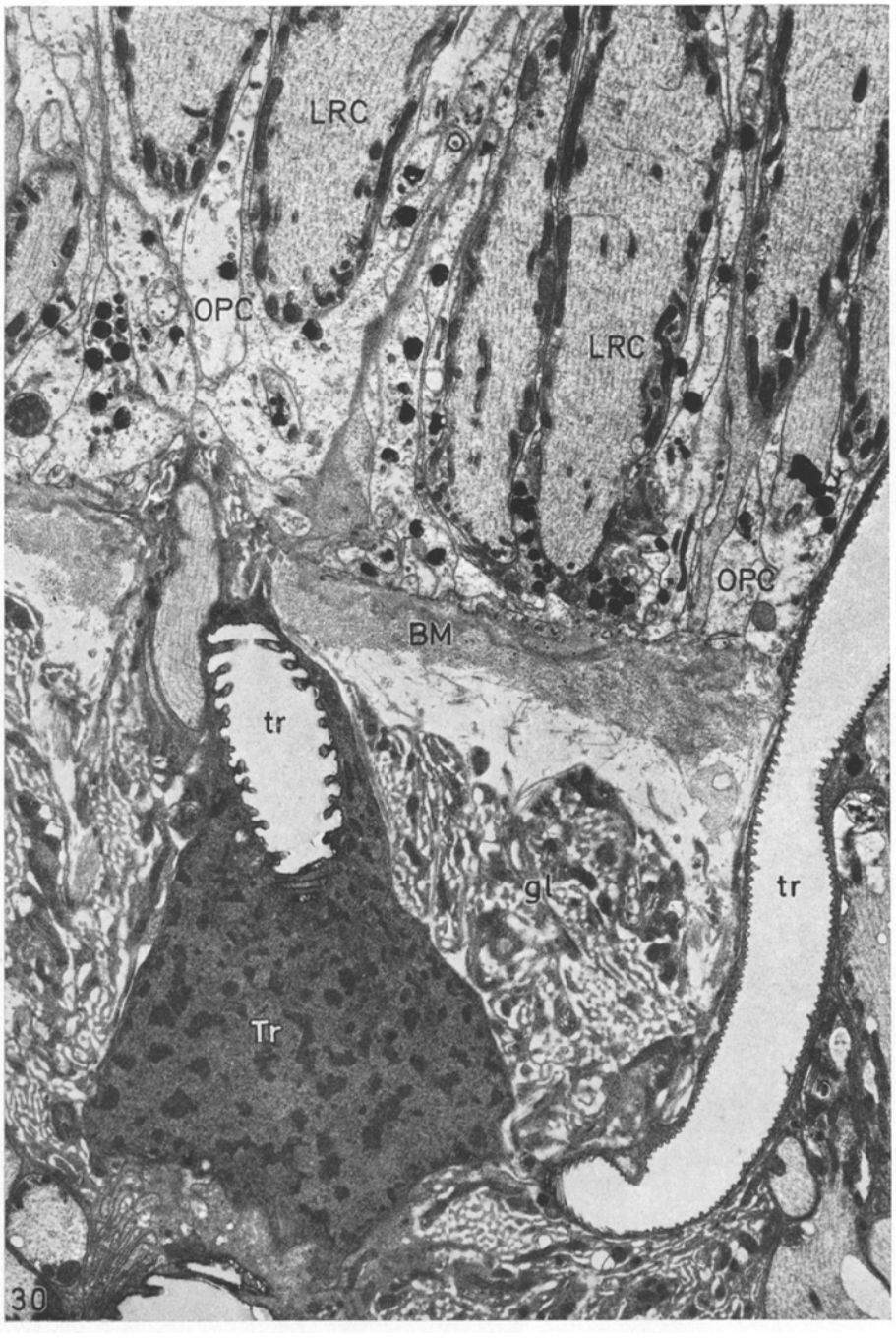


Fig. 30. Slightly oblique section of the basement membrane region. Bundles of the retinula cells axons (*LRC*) are separated from each other by processes of outer pigment cells (*OPC*) adhering to the basement membrane (*BM*). Below the basement membrane, glial (*gl*) cell processes are visible surrounding a large tracheal cell (*Tr*). A tracheal tube (*tr*) is seen piercing the basement membrane towards the retina. × 5,900

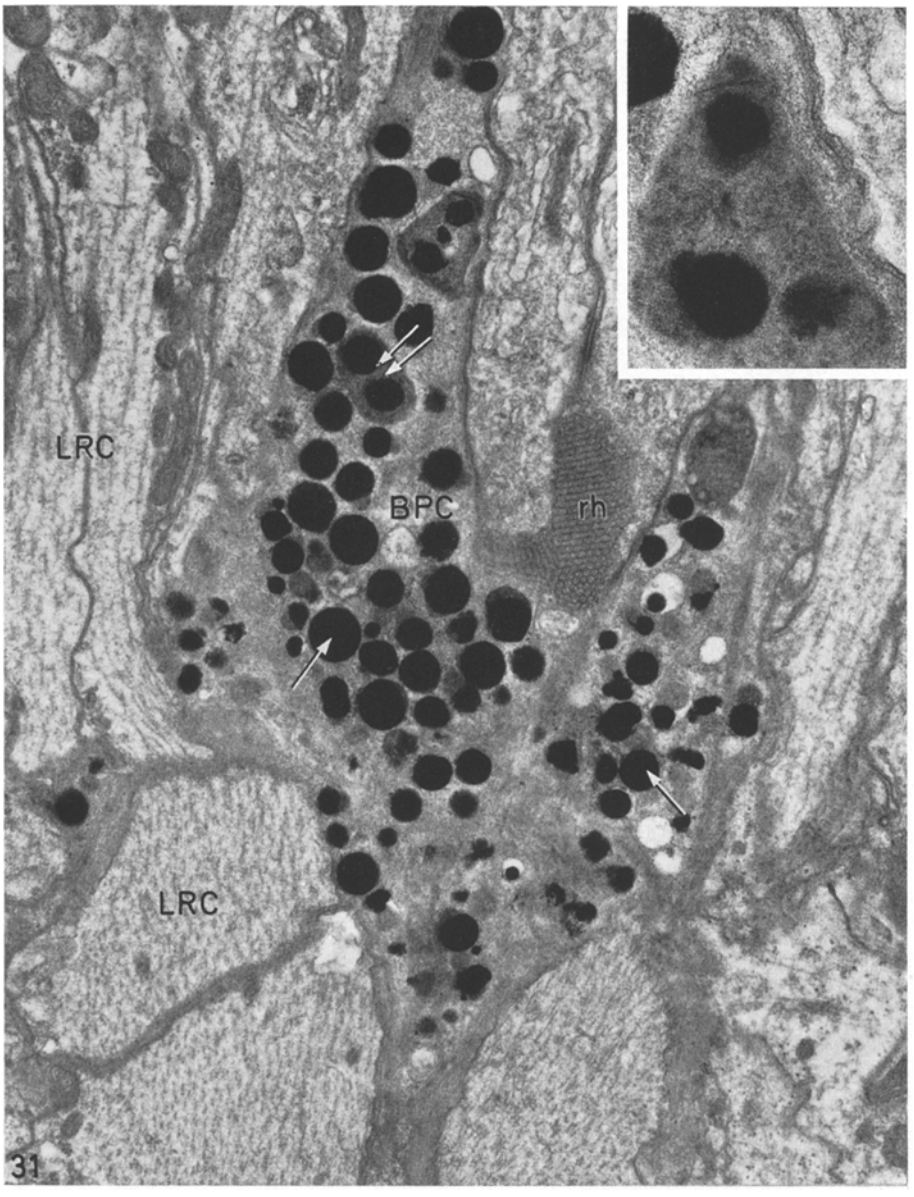


Fig. 31. Oblique section through the base of an ommatidium. At this level, the rhabdome (*rh*) disappears and is replaced by a basal pigment cell (*BPC*). This cell has dark cytoplasm and contains numerous dense pigment granules (arrow), some of them are enclosed in lysosome-like structures (inset). × 14,200, inset × 40,500

be explained by the fact that they sectioned the ommatidia at or above the level of the retinula cell nuclei. At these levels a basal retinula cell like the one described here would not be present and the number of retinula cells therefore 8. Differences between the results of Varela and Porter and those presented here

might well merely be a question of differing interpretations of sections of the ommatidia close to the basement membrane. In fact, Fig. 11 of Varela and Porter's paper is similar to that presented in Fig. 1 of a previous paper on the structure of drone ommatidium (Perrelet and Baumann, 1969b). In both figures, one can distinguish 6 large retinula cells, one small retinula cell, and two axon-like profiles situated at the periphery of the ommatidium. In Fig. 11 of Varela and Porter's paper, the two axons could represent axons of retinula cells separating from the rhabdome at distal level, whereas the retinula cell they called "eccentric" could represent the one which we called "basal", i.e. the ninth cell of the ommatidium[3].

Small or basal retinula cells have been described in several other insect species as for example, *Drosophila* (Waddington and Perry, 1961), *Locusta* (Horridge, 1965), *Lucilia* (Melamed and Trujillo-Cenóz, 1968), *Notonecta* (Horridge, 1968), but it is still unknown whether these cells fullfil a function different to that of the large cells. In the fly *Lucilia* the two small cells (called central cells) have axons which do not synapse in the lamina, unlike those of the large retinula cells. Because of their orthogonal rhabdomeres, they have been thought to be involved in polarized light perception (Melamed and Trujillo-Cenóz, 1968). In the drone, intracellular straining was performed in order to record and localize the response to light of the small retinula cells. These tentatives have so far remained unsuccessful (Bertrand et Perrelet, 1969). Most ommatidia examined in this study stem from the posterior part of the retina. The equatorial and anterior parts of the eye were however the object of a limited number of observations. No differences were observed in the number of retinula cells and in ommatidial organization. The only difference concerns the total length of the ommatidia, which, due to the crescent shape of the eye, are longer in equatorial regions than in the periphery.

The distal extremity of the retinula cells is crowded with cytoplasmic organelles, whereas their proximal part, still bearing rhabdomeres, contains only agranular or subrhabdomeric cisternae and mitochondria. Why there is a large amount of organelles at distal levels (smooth and rough endoplasmic reticulum, free ribosomes, Golgi cisternae), is at present unknown. Pointing out the concomitant presence of numerous granules of accessory pigment, Fahrenbach (1969), however, tentatively suggested that organelles could be involved in accessory pigment metabolism. Differences in cytoplasmic organization of the retinula cells depending on the level of the ommatidium were also reported by Varela and Porter in the worker retina.

Several hypotheses have been put forward as to the possible function of the agranular cisternae (subrhabdomeric) underlying the rhabdome at all levels of the retinula cells. Having noticed that cisternae swell during dark adaptation, Horridge and Barnard (1965), and after them Fahrenbach (1969), postulated the possible optical function of modifying the amount of light trapped in the microvilli by changing the refractive index around the rhabdome. On the other hand, Lasansky (1967) suggested that in the *Limulus* ommatidium, the subrhadomeric

3 *Note added in proof*: In a most recent paper on the lamina of the worker bee, Varela (1970) noted the presence of 9 retinula cell axons originating from one commatidium. The 9th axon in the worker is said to arise from the dichotomy of the "eccentric cell".

cisternae might participate in the transfering of depolarization from the rhabdome to the retinula cell body. In the drone, preliminary observations indicate that agranular cisternae do swell during dark adaptation and contract rapidly after light exposure (Baumann et al., 1967). As in the worker, agranular cisternae were also frequently observed in connection with Golgi stacks and profiles of granular endoplasmic reticulum. The connections between cisternae and Golgi apparatus as well as rough endoplasmic reticulum were interpreted by Varela and Porter (1969) as possible evidence that the cisternae are involved in the metabolism of the photopigment. All these hypotheses have yet to be confirmed by experimental demonstration.

In recording the response to light of pairs of cells in the drone retina with a double micro-electrode, Shaw (1969) demonstrated the presence of electrical coupling between the cells of the same ommatidium. Electrical coupling has been shown to vary from strong to weak with each different cell pair. Guided by electrophysiological data, Shaw hypothesized that the coupling occurs principally through the rhabdomeric microvilli, with or without an accessory coupling of the retinula cells at the base of the ommatidium. Electrical coupling between rhabdomeric microvilli seems possible, since tight junctions were found between the outer leaflets of the microvilli membrane. Well known experiments performed on other tissues have already pointed out the existing correlation between electrical coupling and tight or gap junctions (Barr, Dewey and Berger, 1965; Payton, Bennett and Pappas, 1969). „False" tight junctions have known to be created through the swelling of cells in hypotonic fixative (Schultz and Karlsson, 1965). These can be excluded in our case since the junctions between microvilli were present in the hypertonic (720 mosm) fixative used. Another known cause of artifactual formation of tight junctions, i.e. the dehydration of fixed tissue in acetone (Johnston and Roots, 1967) needs not to be taken into consideration here. As described, only 2 pairs of large cells forming the rhabdome have their microvilli running parallel, i.e. in contact over a long distance; the other cells of the ommatidium only enter in contact at the tips of their microvilli. If the degree of electrical coupling depends on the surface area in contact, this could explain Shaw's finding that certain pairs of cells are strongly coupled, whereas others show weak coupling. The isolation by glial cell processes and fibrillar material of retinula cell axons at the base of the ommatidium does not offer morphological support for electrical coupling at this level.

The finding of tight junctions between rhabdomeric microvilli of the drone retinula cells is in contrast with the results of Varela and Porter (1969), who described definite gaps, 100 Å wide, between all microvilli of the worker ommatidium. Although no electrophysiological data are available to indicate the presence of electrical coupling between the retinula cells of the worker ommatidium, one is entitled to wonder whether the gaps described by Varela and Porter are not due to an inadequate visualization of the outer leaflet of the microvilli membrane, as was shown to be the case in the microvilli of the drone ommatidium (Perrelet and Baumann, 1969a).

The polarized-light sensitivity of the drone retinula cells (Shaw, 1969), could be supported by the morphological evidence of the formation of the rhabdome by the individual rhabdomeres. The rhabdome of the drone, although formed

mainly by six retinula cells, is composed of four parts, each of which is orthogonal with respect to the adjacent one. The association between orthogonal sets of microvilli and polarized-light sensitivity was first pointed out by Waterman (1966), who attributed to the rhabdome the function of a two-channel polarization analyser. This was mainly based on the assumption that photopigment molecules are evenly orientated within the microvilli, thus permitting peak absorption along two perpendicular directions. Indeed, Langer (1965) found dichroic absorption in the open rhabdome of the fly *Calliphora*. Shaw also found the peaks of polarized-light sensitivity separated by 90° in the drone ommatidium.

There are several grounds to believe that the rhabdome is the site of photoreception in arthropod eyes. The rhabdome, formed of successive layers of membranes (microvilli membranes) intercepting the light transmitted by the dioptric apparatus has many morphological features in common with the photoreceptor discs of vertebrate cones and rods. Indeed, frog cones have recently been shown to contain rhodopsin by immuno-fluorescence techniques (Dewey *et al.*, 1969). The bee eye (Goldsmith, 1958), like that of other invertebrates studied so far (Wald, 1968a) contains a retinene-protein complex as photopigment. Moreover, by microspectrophotometry, Langer and Thorell (1966) have succeeded in demonstrating peak absorption in the rhabdome of *Calliphora* at wavelengths identical to those absorbed by rhodopsin in vitro. It therefore seems justified to postulate that drone eye photopigment is also localized in the rhabdome. As already noted, the finely beaded appearence of the photoreceptor membrane could indicate that visual pigment is incorporated in the membrane of the microvilli. Although 3 types of receptors have been detected by stimulation with monochromatic light (Autrum and von Zwehl, 1964), morphological evidence for different types of receptors in the drone retina has not yet been found. A simple explanation is afforded by the fact that the differences in spectral sensitivity of visual cells are due to different photopigments (Wald, 1968), the structure of which at the cellular level is far from being perceptible by actual electron microscopy.

Pigment cells in insect eyes probably serve as light-shields (Goldsmith, 1962; Varela and Porter, 1969), limiting the lateral diffusion of light between neighbouring ommatidia, especially at the level of the dioptric apparatus (largest pigment accumulation). In addition, the pigment cells' position with regards to the retinula cells (primary sensory neurons), as well as their cytoplasmic features, make them resemble glial cells in the central nervous system of vertebrates and invertebrates (Kuffler and Nicholls, 1966).

As expected of apposition eyes of diurnal insects, no gross pigment movement was observed in response to light or dark adaptation, either in corneal pigment cells or in outer pigment cells. This agrees with the findings of Varela and Porter in the worker retina during dark adaptation.

The presence of gap-junctions between the outer pigment cells indicates that these cells could be electrically coupled, like the glial cells in invertebrate and vertebrate nervous systems (Kuffler and Potter, 1964; Kuffler *et al.*, 1966). Intracellular recording in outer pigment cells during light stimulation of the neighbouring ommatidia, revealed no coupling between retinula cells and outer pigment cells (Bertrand and Perrelet, 1969). This was as expected, in view of the

continuous extracellular space, 100—200 Å wide, existing between the two cell types. These morphological features, as well as the demonstrated penetration of large particles (ferritin) in the rhabdome, indicate that the exchanges between the periphery and the center of the ommatidium could occur through extracellular channels between pigment cells, in a similar way as those between glial cells and neurons of the central nervous system of invertebrates (Nicholls and Kuffler, 1964). This hypothesis is also supported by the rapid modification of the response to light recorded in retinula cells when the ionic composition of the bathing medium is changed (Fulpius and Baumann, 1969).

References

Autrum, H., Zwehl, V. von: Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. Z. vergl. Physiol. **48**, 357—384 (1964).

Barr, L., Dewey, M. M., Berger, W.: Propagation of action potentials and the structure of the nexus in cardiac muscle. J. gen. Physiol. **48**, 797—823 (1965).

Baumann, F.: Slow and spike potentials recorded from retinula cells of the honey bee drone in response to light. J. gen. Physiol. **52**, 855—875 (1968).

— Perrelet, A., Fulpius, B.: Etude fonctionelle et morphologique de la cellule rétinienne du faux-bourdon au cours de l'adaptation à la lumière et à l'obscurité . Helv. Physiol. Acta **25**, CR 163 (1967).

Bertrand, D., Perrelet, A.: Localisation par coloration intracellulaire de divers types de réponse à la lumière dans l'oeil du faux-bourdon. J. Physiol. (Paris) (1969) (in press).

Blasie, J. K., Dewey, M. M., Blaurock, A. E., Worthington, C. R.: Electron microscope and low-angle X-ray diffraction studies on outer segment membranes from the retina of the frog. J. molec. Biol. **14**, 143—152 (1965).

Butenandt, A., Biekert, E., Linzen, B.: Über Ommochrome. XIII. Isolierung und Charakterisierung von Omminen. Hoppe-Seylers Z. physiol. Chem. **312**, 227—236 (1958).

Chliamovitch, Y. P.: Effets du potassium sur le potentiel de repos de la cellule rétinienne du faux-bourdon. BS Thesis, Faculty of Sciences, University of Geneva (1970).

Dewey, M. M., Davis, P. K., Blasie, J. K., Barr, L.: Localization of rhodopsin antibody in the retina of the frog. J. molec. Biol. **39**, 395—405 (1969).

Drochmans, P.: Morphologie du glycogène. J. Ultrastruct. Res. **6**, 141—163 (1962).

Fahrenbach, W. H.: The morphology of the eyes of *Limulus*. II. Ommatidia of the compound eye. Z. Zellforsch. **93**, 451—483 (1969).

Farquhar, M. G., Palade, G. E.: Junctional complexes in various epithelia. J. Cell Biol. **17**, 375—412 (1963).

Fernández-Morán, H.: The fine structure of vertebrate and invertebrate photoreceptors as revealed by low-temperature electron microscopy. In: The structure of the eye, ed. by G. K. Smelser, p. 521—556. New York: Academic Press 1961.

Fulpius, B., Baumann, F.: Effects of Na, K and Ca ions on slow and spike potentials in single photoreceptor cells. J. gen. Physiol. **53**, 541—561 (1969).

Goldsmith, T. H.: The visual system of the honey bee. Proc. nat. Acad. Sci. (Wash.) **44**, 123—126 (1958).

— Fine structure of the retinulae in the compound eye of the honey bee. J. Cell Biol. **14**, 489—494 (1962).

— The visual system of insects. In: The physiology of insecta, ed. by M. Rockstein, p. 397—462. New York: Academic Press 1964.

Hadjilazaro, B.: Afterpotentials of the drone retinula cells and mechanisms of their generation. Ph. D. Thesis, University of Geneva (1970).

Horridge, G. A.: The retina of the locust. In: The functional organization of the compound eye. Wenner-Gren Symposium, ed. by C. G. Bernhard, p. 513—541. London: Pergamon Press 1966.

— A note on the number of retinula cells of *Notonecta*. Z. vergl. Physiol. **61**, 259—262 (1968).

Horridge, G. A., Barnard, P. B. T.: Movement of palisade in locust retinula cells when illuminated. Quart. J. micr. Sci. **106**, 131—135 (1965).
Johnston, P. V., Roots, B. I.: Fixation of the central nervous system by perfusion with aldehydes and its effect on the extracellular space as seen by electron microscopy. J. Cell Sci. **2**, 377—386 (1967).
Karnovsky, M. J.: Simple methods for "staining with lead" at high pH in electron microscopy. J. biophys. biochem. Cytol. **11**, 729—732 (1961).
— The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. **35**, 213—236 (1967).
Kuffler, S. W., Nicholls, J. G.: The physiology of neuroglial cells. Ergebn. Physiol. **57**, 1—90 (1966).
— — Orkand, R. K.: Physiological properties of glial cells in the central nervous system of amphibia. J. Neurophysiol. **29**, 768—787 (1966).
— Potter, D. D.: Glia in the leech central nervous system: physiological properties and neuron-glia relationship. J. Neurophysiol. **27**, 290—320 (1964).
Langer, H.: Nachweis dichroitischer Absorption des Sehfarbstoffes in den Rhabdomeren des Insektenauges. Z. vergl. Physiol. **51**, 258—263 (1965).
— Thorell, B.: Microspectrophotometry of single rhabdomeres in the insect eye. Exp. Cell Res. **41**, 673—677 (1966).
Lasansky, A.: Cell junctions in ommatidia of *Limulus*. J. Cell Biol. **33**, 365—383 (1967).
Locke, M.: The structure and formation of the integument in insects. In: The physiology of insecta, ed. by M. Rockstein, p. 379—470. New York: Academic Press 1964.
Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409—414 (1961).
McManus, J. F. A.: Histological demonstration of mucin after periodic acid. Nature (Lond.) **158**, 202 (1946).
Melamed, J., Trujillo-Cenóz, O.: The fine structure of the central cells in the ommatidia of dipterans. J. Ultrastruct. Res. **21**, 313—334 (1968).
Millonig, G.: Advantages of a phosphate buffer for OsO_4 solutions in fixation. J. appl. Physics **32**, 1637 (1961).
Mira-Moser, F.: Histophysiologie expérimentale de la fonction thyréotrope chez le crapaud *Bufo bufo* L. Arch. Anat. (Strasbourg) **52**, 88—182 (1969).
Naka, K., Eguchi, E.: Spike potentials recorded from the insect photoreceptor. J. gen. Physiol. **45**, 663—680 (1962).
Nicholls, J. G., Kuffler, S. W.: Extracellular space as a pathway for exchange between blood and neurons in the central nervous system of the leech: ionic composition of glial cells and neurons. J. Neurophysiol. **27**, 645—671 (1964).
Nilsson, S. E.: The ultrastructure of the receptor outer segments in the retina of the leopard frog (*Rana pipiens*). J. Ultrastruct. Res. **12**, 207—231 (1965).
Orci, L., Junod, A., Pictet, R., Renold, A. E., Rouiller, Ch.: Granulolysis in A cells of endocrine pancreas in spontaneous and experimental diabetes in animals. J. Cell Biol. **38**, 462—466 (1968).
Payton, B. W., Bennett, M. V. L., Pappas, G.: Permeability and structure of junctional membranes at an electrotonic synapse. Science **166**, 1641—1643 (1969).
Perrelet, A.: La structure fine de l'oeil du faux-bourdon *Apis mellifera*. M. D. Thesis, University of Geneva (1969).
— Baumann, F.: Evidence for extracellular space in the rhabdome of the honey bee drone eye. J. Cell Biol. **40**, 825—830 (1969a).
— — Presence of three small retinula cells in the ommatidium of the honey bee drone eye. J. Microscopie 8, 497—502 (1969b).
Phillips, E. F.: Structure and development of the compound eye of the honey bee. Proc. Acad. nat. Sci. Phil. **57**, 123—157 (1905).
Phillips, M. J., Unakar, N. J.: Glycogen depletion in the newborn rat liver. An electron microscopic and electron histochemical study. J. Ultrastruct. Res. **18**, 142—165 (1967).
Revel, J. P.: Electron microscopy of glycogen. J. Histochem. Cytochem. **12**, 104—114 (1964).
— Karnovsky, M. J.: Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J. Cell Biol. **33**, C_7—C_{12} (1967).

Röhlich, P., Törö, I.: Fine structure of the compound eye of *Daphnia* in normal, dark- and strongly light-adapted state. In: Eye structure. II Symp., ed. by J. W. Rohen, p. 175—186. Stuttgart: Schattauer 1965.

Sabatini, D. D., Bensch, K., Barrnett, R. J.: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17**, 19—58 (1963).

Sanchez, D. S.: Sobre la existencia de un aparato tactil en los ojos compuestos de las abejas. Trab. Lab. Invest. Biol. Madrid **18**, 207—244 (1920).

Schultz, R. L., Karlsson, U.: Fixation of the central nervous system for electron microscopy by aldehyde perfusion. II. Effects of osmolarity, pH of perfusate and fixative concentration. J. Ultrastruct. Res. **12**, 187—206 (1965).

Seitz, G.: Der Strahlengang im Appositionsauge von *Calliphora erythrocephala*. Z. vergl. Physiol. **59**, 205—231 (1968).

Shaw, S. R.: Interreceptor coupling in ommatidia of drone honey bee and locust compound eyes. Vision Res. **9**, 999—1029 (1969).

Shoup, J. R.: The development of pigment granules in the eyes of wild type and mutant *Drosophila melanogaster*. J. Cell Biol. **29**, 223—249 (1966).

Smith, R. E., Farquhar, M. G.: Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland. J. Cell Biol. **31**, 319—347 (1966).

Varela, F. G.: Fine structure of the visual system of the honey bee. II. The lamina. J. Ultrastruct. Res. **31**, 178—194 (1970).

Varela, F. G., Porter, K. R.: Fine structure of the visual system of the honeybee. I. The retina. J. Ultrastruct. Res. **29**, 236—259 (1969).

Venable, J. H., Coggeshall, R. E.: A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. **25**, 407—408 (1965).

Waddington, C. H., Perry, M. M.: The ultrastructure of the developing eye of *Drosophila*. Proc. roy. Soc. B **153**, 155—173 (1961).

Wald, G.: Single and multiple visual systems in arthropods. J. gen. Physiol. **51**, 125—156 (1968a).

— Molecular basis of visual excitation. Science **162**, 230—239 (1968b).

Waterman, T. H.: Systems analysis and the visual orientation of animals. Amer. Scientist **54**, 15—45 (1966).

White, R. H.: The effect of light and light deprivation upon the ultrastructure of the larval mosquito eye. II. The rhabdom. J. exp. Zool. **166**, 405—426 (1967).

Wigglesworth, V. B.: Principles of insect physiology. London: Menthuen & Co. Ltd. 1965.

Wolken, J. J., Florida, R. G.: The eye structure and optical system of the crustacean copepod, *Copilia*. J. Cell Biol. **40**, 279—285 (1969).

Wood, R. L.: Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy. J. biophys. biochem. Cytol. **6**, 343—351 (1959).

Dr. A. Perrelet
Institut d'Histologie
et d'Embryologie
Ecole de Médecine
CH-1211 Genève 4