Movement of palisade in locust retinula cells when illuminated

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With 4 plates

Summary
In dark-adapted eyes cisternae of the endoplasmic reticulum collect near the rhabdom and form around it a functionally significant region 2 to 4 μ across, of lowered refractive index. In light-adapted eyes this structure is dispersed as vacuoles in the cytoplasm, and instead mitochondria are crowded round the rhabdom. Onion bodies up to 8 μ in diameter occur in the retinula cell cytoplasm.

Introduction
The structure of the ommatidium in the locust is typical of the fused rhabdom apposition eyes already described in grasshoppers (Jörschke, 1914; Fernández-Morán, 1958), the cockroach (Wolken and Gupta, 1961), and the honeybee (Goldsmith, 1962). A further anatomical study on the locust is justified by the recent physiological results and theories concerning the retinula cells and optics of vision in this animal. Recordings with intracellular electrodes can be made from the retinula cells, a fact which makes possible many exact physiological and optical measurements. We sought an effect of light on the ultrastructural detail of these cells. A fuller account of the structure of the retina is in preparation.

Methods
The locusts (Locusta migratoria) were reared in a cage containing one 60-watt bulb which was never switched off. The possibility of a circadian rhythm interfering with the experiments is therefore ignored. For electron microscopy the animals were decapitated and segments were immediately cut from the eye in a known orientation and dropped into fixative. Light-adapted eyes were taken from animals straight out of the cage with the lamp; dark-adapted eyes came from animals which had been in total darkness for 24 h and were operated and fixed in dim red light. Observations were made on twenty eyes in each state. The fixative consisted of a mixture of equal parts of Hoyle's locust saline and 2 per cent osmic acid. Blocks were dehydrated in an acetone series, embedded in Araldite, sectioned, and stained with lead. Comparisons between dark- and light-adapted eyes have been based upon material which has been treated by a constant procedure. Sections cut in Araldite were stained with toluidine blue for light microscopy.

Results

In the locust there are one or two eccentric cells and six or seven normal retinula cells, always making a total complement of eight cells with axons per ommatidium. The eccentric cells, on the dorsal and ventral sides of the ommatidium, have a flat distal process which extends between their neighbouring retinula cells and is pressed against the rhabdom for a distance of about 20 to 30 μ in the region below the level of the nuclei of the typical retinula cells. The tip of this process of the eccentric cell has a rudimentary rhabdomere, having a few very short tubules. The structures and changes to be described do not apply to the eccentric cells.

In the region immediately next to the rhabdom, in the cytoplasm of the retinula cells, there is a clear zone 2 to 4 μ across in dark-adapted eyes (figs. 1 and 2). This is the 'Schaltzone' of the old light microscopists. In the electron microscope the zone consists of a group of vacuoles which can be seen in figures of retinula cells of Dissosteira, Anax and Apis published by the above-mentioned authors, but tentatively called tracheoles by Fernández-Morán (1958). Each vacuole is derived from endoplasmic reticulum (ER), as shown by abundant instances in which the central space is continuous with the space between the paired membranes of the endoplasmic reticulum. The vacuole is surrounded by a membrane which is similar to a single endoplasmic reticulum membrane. It is proposed to call these the endoplasmic cisternae or lacunae, and, where they collect together in dark-adapted eyes, to call the loose structure round the rhabdom the palisade. In dark-adapted eyes the palisade is crossed by thin cytoplasmic bridges connecting the rhabdomere with the rest of its cell.

In light-adapted eyes the palisade has disappeared and its place is taken by large numbers of mitochondria which have moved close to the rhabdomere from the cytoplasm of the remainder of the cell. On the other hand the lacunae are now distributed in the cytoplasm, sometimes in a pattern radiating from the centre of the ommatidium. Typical sections can be compared in figs. 3 and 4. The changes are greater in the peripheral than in the deeper levels of the retina and are most obvious in the area near the tip of the cone.

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Figs. 1 and 2. Light micrographs of the peripheral ends of the retinula cells from thin sections of material embedded in Araldite and stained with toluidine blue. The resolution of the palisade shows that it is sufficiently large, compared with the wavelength of light, to exert an optical effect. Note small pigment grains within the retinula cells and larger ones within the glial cells which surround them.

Fig. 1, transverse section at the level of the retinula cell nuclei.

Fig. 2, longitudinal section through the tips of two cones at their widest points. More pigment grains are visible because the section is thicker than that shown in fig. 1.

Fig. 3. Transverse section through an ommatidium at the level of the retinula cell nuclei in a light-adapted eye. Mitochondria surround the nucleus and ER cisternae are scattered through the cytoplasm.

c, cone; e, extension of cone as five fibrous threads between the retinula cells; er, endoplasmic reticulum; l, lacunae in cytoplasm; g, glial cell cytoplasm; m, mitochondria; n, nucleus of retinula cell; p, palisade; r, rhabdom.
FIGS. 1-2

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FIG. 4

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FIG. 5
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where the illumination can be expected to be greatest. These structures have the appearance of being filled with fluid, and comparisons of cross sections show that the volume of the lacunae plus palisade remains approximately constant. Therefore it seems reasonable to suppose that they move in bulk. To ensure that transverse sections were from comparable depths in the retina, sections were taken at the level of the retinula cell nuclei, where the eccentric cell is not seen. Changes in the position of the small pigment grains of the retinula cells and the larger ones of the surrounding glial pigment cells were not observed.

In sections examined with the light microscope the palisade appears as a faintly reticulate zone around the rhabdom. It is therefore large enough to exert an influence on the optics of the eye, and movements of the palisade assume some importance in relation to the theory that the rhabdom acts as a light guide.

Comparisons of the refractive index of the region immediately surrounding the rhabdom were attempted in dark- and light-adapted eyes using an interference microscope. Measurements could not be made with fresh or frozen sections, and therefore, as a less satisfactory alternative, material was embedded in wax which was removed on the slide and the sections were immersed in ethylene glycol. It must be pointed out that the lipids are removed. In dark-adapted eyes there is a decreased phase retardation in the neighbourhood of the rhabdom as compared with sections of light-adapted eyes cut at the same thickness setting on the microtome. To avoid errors caused by differences in thickness, measurements are given as comparisons with the rhabdom of the same section. In ten measurements on three different dark-adapted eyes the retardation in the palisade ranged from 82 to 90 per cent of the retardation in the rhabdom at a wavelength of 5164 Å. In light-adapted eyes the palisade zone is not resolved and the cytoplasm immediately surrounding the rhabdom has a refractive index which is not distinguishable from that of the rhabdom. Evidently the mitochondria, which are certainly electron dense, have a high refractive index in the light microscope and might be mistaken for part of the rhabdom.

Rhabdoms of dark- and light-adapted eyes have been examined for differences in dimensions, but in such work it is difficult to obtain comparable random samples by electron microscopy. Results so far show no difference in the areas of rhabdoms in cross-sections of light- and dark-adapted eyes at the same level. In light-microscope studies of other insect eyes, other

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**Fig. 4.** Transverse section as in fig. 3, but of a dark-adapted eye. The palisade surrounds the rhabdom and the mitochondria are scattered. e, extension of cone as four fibrous threads between the retinula cells; er, endoplasmic reticulum; l, lacuna in cytoplasm; g, glial cell cytoplasm; m, mitochondria; n, nucleus of retinula cell; p, palisade; r, rhabdom.

**Fig. 5.** Onion body within the cytoplasm of a retinula cell. Although consisting of membrane pairs these are smoother than those of the typical endoplasmic reticulum, and the onion body is enormous compared with the Golgi complexes. cm, cell membrane; e, extension of cone, containing fibres cut across; er, typical rough endoplasmic reticulum; g, Golgi membranes forming a complex of typical size and appearance; m, mitochondrion.
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authors have found that the rhabdoms increase in length in the dark (Umbach, 1934; Sato et al., 1957; Reis, 1960). The measurements on the locust rhabdoms, however, show an increase in the variability of the cross-sectional area at the 0.5 per cent level of significance. The diameters of microvilli of the rhabdomeres were similarly compared in dark- and light-adapted eyes but no consistent difference has so far appeared. The results show, however, a greater variability of tubule diameter in dark-adapted eyes at the 0.5 per cent level of significance.

Onion bodies with a characteristic spherical laminate structure occur in the cytoplasm of the peripheral ends of the retinula cells (fig. 5). They consist of ten to thirty concentric membranes, and are 4 to 8 \( \mu \) in diameter, large enough for histochemical work. They have the same structure in transverse and longitudinal sections. The outermost layers frequently have small electron-dense particles resembling those on the typical rough endoplasmic reticulum. No differences have been found in onion bodies of light- and dark-adapted eyes.

Discussion

Although absolute measurements of the refractive indices are required before the optics of the locust eye can be elucidated, the limited measurements available show that the palisade must increase the acceptance angle because of its low refractive index and large size. The palisade will tend, however slightly, to keep light within the rhabdom by internal reflection. The longitudinal section (fig. 2) suggests how the light reaches this region in the first place. The size of the narrowest aperture at the tip of the cone appears to be large enough to allow conclusions to be drawn from ray diagrams.

Changes in the sensitivity of the insect eye to light with dark-adaptation have been known from behavioural tests or measurements of the electroretinogram for many years. In their general accounts both Prosser (1950) and Dethier (1963) point out that while these changes may be linked with the movement of pigment grains, there are instances where this is not so. For example the eye of the water beetle Dytiscus shows a diurnal rhythm with a thousandfold increase in sensitivity at night without an accompanying pigment migration. In the locust, illumination causes little pigment movement, although, as in the bee (Goldsmith, 1963), there is an increase in sensitivity of about three log units on dark-adaptation.

The formation of a palisade in dark-adapted eyes would bring about an increased sensitivity because more light would be kept within the rhabdom. However, it is hardly to be expected that the large changes in sensitivity can be explained in this way. Pigment movements are negligible in the locust eye and have been eliminated as a possible cause of the large changes also in the honeybee (Goldsmith, 1963).

No mechanism of the palisade and mitochondria movements is suggested by the present observations. The onion bodies have been introduced as new possible photoreceptor structures in the cytoplasm and they suggest several
hypotheses. Similar arrays of membranes have been found in some vertebrate eyes. Suggestions have been advanced elsewhere that such structures may be receptor organelles for pigment migration or similar cytoplasmic changes (see Bernstein, 1961) but only circumstantial evidence lends credence to these ideas.

References


