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Cone mosaic in a teleost retina: No difference between light and dark adapted states¹

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Summary. The mosaic arrangement of retinal cones in the eye of the African cichlid fish, *Haplochromis burtoni*, is the same in both light and dark adapted states. This is in contrast to Kunz' claim² that the retinal mosaic pattern changes from a square to a row type during dark adaptation, in the guppy (*Poecilia reticulata*). Kunz' histological procedure may account for this difference in results.

Cone photoreceptors in many teleost retinas are arranged in a highly regular mosaic pattern³⁻⁶. 2 basic types of mosaic patterns have been described; 'square' and 'row' types. In both patterns, a row of double or twin cones alternates with a row of single cones. The patterns differ in that in one case the double (twin) cones are arranged so that lines through the centers of each pair are at right angles with respect to one another ('square' pattern) whereas in the other case the axes of the double cones are parallel to one another ('row' pattern) (fig. 1, a). The 'square' mosaic (fig. 1, b) is thought to be associated with fish which are highly dependent on vision³. In some species, both patterns exist in different regions of adult retina^{7,8} or occur at different times during ontogeny⁹⁻¹¹. Rods are interspersed in the cone arrays throughout the extent of the retina¹².

In the African cichlid fish, *Haplochromis burtoni*, a highly ordered square type mosaic is present¹³. The chromatic organization of these cones, as determined by vital staining with nitro-blue tetrazolium di-formazan, is an alternating symmetry which serves to maximize the chromatic resolution available to the animal¹². This is consistent with behavioral^{14,15}, developmental¹⁶, and neuroanatomical¹⁷ evidence that vision is the primary sensory input for *H. burtoni*.

In a recent report, Kunz² suggested that the cone mosaic, as reflected in outer segment organization in the guppy (*Poecilia reticulata* P.) changes from a square to a row type during dark adaptation. She removed adult fish retinas by microdissection in Ringer's solution with or without calcium and examined the tissue using light or electron microscopy. At the level of the outer segments, her micrographs show an apparent change in mosaic type from double cone zig-zag rows (fig. 1, b) to parallel rows (fig. 1, a). She suggests that this change supports the hypothesis that zig-zag rows of double cones are more suitable for high acuity vision and parallel rows more suitable for low-level light detection. If this hypothesis were true, such a change in mosaic type should occur in other teleost species dependent on vision during dark adaptation. For this reason, we examined the cone mosaic in the retina of *H. burtoni* under conditions of light and dark adaptation.

Materials and methods. Adult male *Haplochromis burtoni* (7-8 cm long) bred from wild-caught fish¹⁴ were used for the experiments. The animals were maintained at 12:12 light-dark cycle characteristic of their natural habitat in central Africa¹⁴. Animals were anesthetized by immersion in tricaine methanesulphonate (MS222, Sandoz) at 03.00 h and one eye removed, and the procedure repeated at 15.00 h and the other eye removed. This allowed us to compare 2 eyes from the same animal, differing only in the time of day when they were prepared for histology. Enuclation during the dark portion of the light-dark cycle was performed using an infrared viewing device (FJW, Mt. Prospect, Ill.) so that there was no significant illumination below 750 nm, which is well above the photopigment sensitivity limit¹³. Following enucleation, the lenses were removed and the eyecups were immediately immersed in fixative (1% paraformaldehyde, 2.5% glutaraldehyde, 3% sucrose in 0.06 M PO₄ buffer) with dorsal margin of the eye

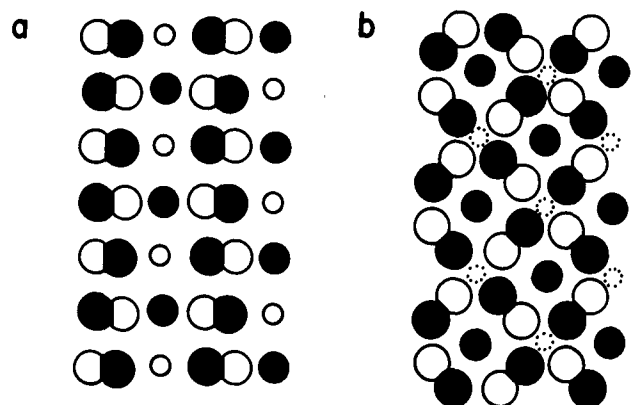


Figure 1. a A sketch of the 'row' type of cone pattern. The rows of single cones alternate with rows of double cones which are oriented so that lines drawn through the twin cones are parallel. b 'Square' type of cone pattern. The rows of double cones are oriented so that lines drawn through the twin cones are perpendicular. Dotted lines indicate single cones which are not present in *H. burtoni* retina.

marked to allow orientation during sectioning. The eyecups were post-fixed (1% osmium, 3% sucrose in 0.06 M PO_4 buffer at pH 7.3), washed, and stored overnight in 0.2 M cacodylate buffer, pH 7.3. Following washing and dehydration, the eyecups were embedded in epon. Sections were stained with 0.05% toluidine blue and sectioned at 3 μm intervals.

To examine the retinal mosaic, tangential sections through the margin of the eye were used, and to confirm the state of retinomotor movement, sections near the midline were examined.

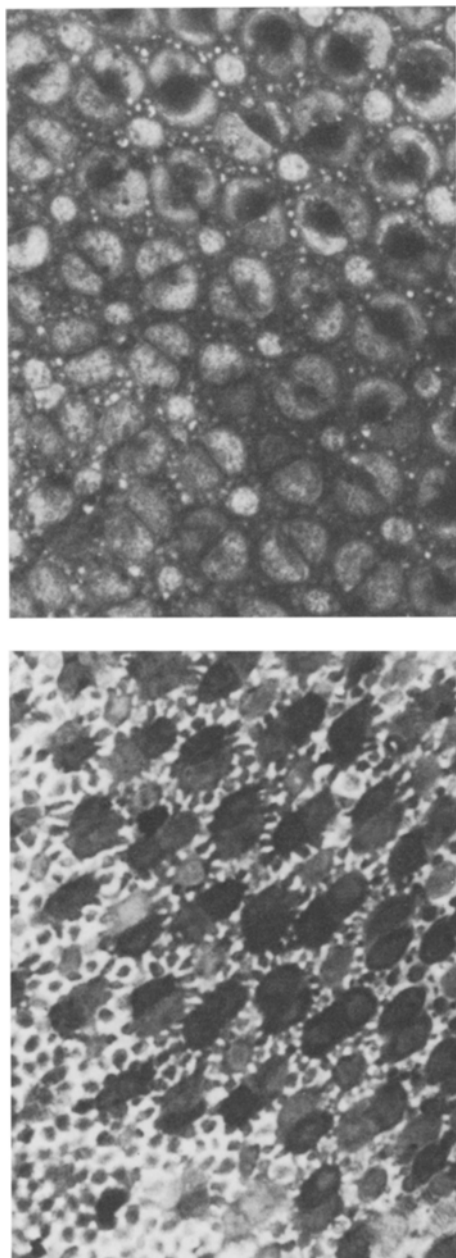


Figure 2. *a* Cone photoreceptor mosaic in the African cichlid fish, *Haplochromis burtoni*. Tangential section at the level of the outer segment. This retinal tissue was prepared from an eye removed while it was light-adapted (see text). *b* Cone photoreceptor mosaic prepared during dark adaptation. This eye is from the fish as in figure 2, *a*, removed 12 h earlier (see text for details).

Results and discussion. Viewing the retinal sections under a light microscope revealed that the square mosaic arrangement of retinal cones is present in both day and night adapted states of the retinae (fig. 2, *a* and *b*). In light-adapted retinae (fig. 2, *a*), the square mosaic pattern is easier to visualize because the outer segment of the single cone can be seen in the same plane of section as the outer segments of the twin cone members. In the dark-adapted retina (fig. 2, *b*), the square pattern is also evident. The square matrix found in the dark-adapted animal is slightly skewed but nonetheless still is distinctly of the type shown schematically in figure 1, *b*.

In summary, there is no difference in the mosaic pattern between retinae which are light-adapted and those which are dark-adapted. What could account for the differences between these results and those of Kunz? First, it could be that this difference reflects a true species difference between the African cichlid fish, *Haplochromis burtoni*, and the guppy, *Poecilia reticulata*. This seems unlikely, given the high levels of similarity in numerous other aspects of teleost fish retinae⁵. Although this possibility cannot be excluded by the present evidence, a second, more parsimonious explanation seems appropriate. The preparation of the tissue can greatly affect the stability and apparent order within retinal tissue. In addition, the plane of section can alter the appearance of the mosaic. For comparison, we prepared retinae as above, using Bouin's fixative rather than the paraformaldehyde-glutaraldehyde combination. Although the results are the same as those described here, the amount of 'wobble' in the matrix pattern is significantly larger. This is reasonable because Bouin's fixative penetrates more slowly. The cone outer segments are fragile and may easily be disrupted by the procedures used for their examination. It seems most likely that the use of calcium-free Ringer's solution to expedite dissection, and whole retina dissection, contribute to the apparent appearance of row-type matrices in a guppy retina in Kunz' work. By fixing the tissue immediately following sacrifice, embedding and sectioning, it seems reasonable that the results we obtained here could be replicated in the guppy. We have attempted to obtain whole-mounted retinae in the fashion described² and found an obvious increase in the irregularity of the cone mosaic pattern. This may easily be attributed to the tissue preparation and not to an intrinsic property of the retina itself.

Teleost fish probably have mosaic retinae because the dioptric power of the eye is vested entirely in the spherical fish lens, which favors the retina being close to that lens¹². To achieve adequate visual acuity in such a retina, the photoreceptor elements must be closely spaced and presumably the packing optimized. As the eyes enlarge, the spacing between cones can increase without loss of acuity, because the larger lens produces a larger image. The 'square' matrix is better suited for visual discrimination, assuming the members of the twin can function independently, since their orthogonal relationship increases the inherent range of sensitivity of the photoreceptors. That is, a line moving across the retina which is parallel to the long axis of one pair of cones will intersect the next pair at right angles to its direction of movement, making the line easier to localize.

It seems unlikely that teleost fishes would change their mosaic type through rotation of the cone outer segments in the dark. The outer segments are surrounded by pigment epithelium and almost certainly do not collect much light.

The apparent change in mosaic type during dark adaptation may more parsimoniously be ascribed to histological preparation which relieves the need for postulating intricate reasons or mechanisms to achieve rotation of the twin cones during retinomotor activity.

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Fenestrated capillaries in 'venous patch' adventitia

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Summary. Fenestrated capillaries in venous patch adventitia are seen during the 2nd week after microsurgery. The fenestrae contain diaphragms. These capillaries are possibly transitory structures which appear during injury cicatrization. The importance of these capillaries during transcappillary exchange in injured tissue is discussed.

When grafted to animal arteries less than 1 mm in external diameter, the venous patch graft provides the opportunity to study, among other problems, the morphological changes in patch vasa vasorum capillaries. Tight junctions open and pinocytotic vesicles increase in number, whereas the basal lamina remains unchanged². 6 days after surgery, the most external part of the venous patch wall presents new capillaries formed by the proliferation of intact capillaries of arterial vasa vasorum, situated close to the suture². Endothelial cells exhibit tight junctions characteristic of mature endothelium³. The capillaries ramify throughout the adventitia, usually stopping at the external border of the media. During the 1st 3 weeks after surgery, capillaries of the venous patch vasa vasorum greatly increase in

number and these are lined by a continuous endothelium with an uninterrupted basal lamina. 2 weeks after surgery, the venous patch and the host artery intima presented a muscular hyperplasia² and patch revascularization occurred at the same time. In order to correlate hyperplasia and revascularization of the patch, we employed the venous patch technique and, since revascularization is evident 2 weeks after surgery, we studied animals at the period and we found (unexpected) fenestrated capillaries in the venous patch wall. The purpose of the present study was to analyze such types of capillaries.

Material and methods. Experiments were performed on 16 male Wistar rats weighing around 250 g. All animals had free access to standard rat chow and water. Nembutal (30 mg/kg) was injected i.p. With the help of an operating microscope, a 10–12 mm long segment of the saphenous vein was extirpated and longitudinally incised on a rectangular strip ('venous patch') that was immediately placed in a saline solution. The right common carotid artery was then dissected and clamped as caudally and cranially as possible with microvascular clip, and a 5–8 mm longitudinal ar-

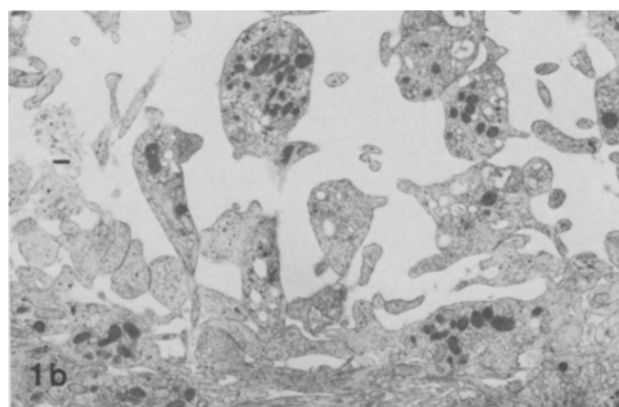
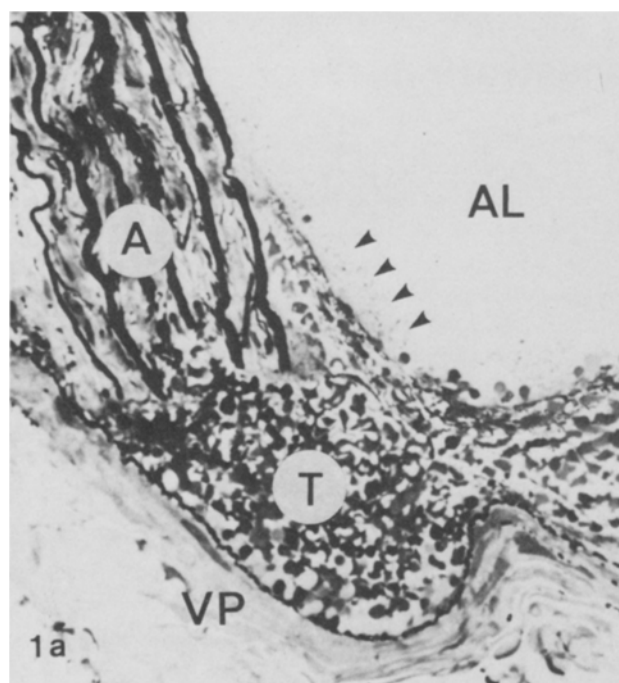


Figure 1. *A* Confluence area between artery (A) and venous patch (VP). Arrows point out the platelets, T, red thrombus; AL, Arterial lumen. Rat in the 1st day after surgery. $\times 1380$. *B* Platelet aggregation in the suture line. 1st h after surgery. $\times 5700$.