Cone Droplets of Mitochondrial Origin in the Retina of *Fundulus heteroclitus* (Pisces: Cyprinodontidae)

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**Summary.** Cone oil droplets in the retina of the adult killifish, *Fundulus heteroclitus*, were investigated by light and electron microscopy. The droplets appear to be formed by mitochondrial transformations in a vitreal-scleral gradient, and the position of the mature droplet is in the ellipsoidal region nearest the cone outer segment. The mode of mitochondrial maturation and the ultrastructural configuration of the droplet differ from one type of cone to another. The latter finding is discussed in terms of the functional significance of these droplets, and the occurrence of retinal oil droplets among the teleosts is briefly reviewed.

**A. Introduction**

The retina of some cyprinodontid and poecilid fishes is characterised by the presence of a dense globular mass at the scleral end of some cone ellipsoids, when stained for light microscopy. This peculiar feature has been observed in the principal member of the double cone of the guppy, *Lebistes reticulatus* (Müller, 1952; Lang, 1965) and two poecilids, *Xiphophorus helleri* and *Mollienisia* sp. (Engström, 1963). In the killifish, *Fundulus heteroclitus*, this globular body is found in the single as well as the double cones (Butcher, 1938), the latter possessing a dark granular mass in one component and a pale one in the other. However, this globule is apparently absent in the retina of another cyprinodontid fish, *Panchax lineatus* (Engström, 1963).

The oil droplet, which is usually found at the scleral end of the cone ellipsoid in some fishes (coelacanth, lungfish, sturgeon, bowfin, lamprey), anurans, reptiles, birds and non placental mammals (Walls, 1942), was long considered absent in the teleosts (see Munk, 1968) until Berger (1966) noted it in the guppy at the ultrastructural level. More recently its occurrence in teleost retinas was still disputed (Crescitelli, 1972; Muntz, 1972; Rodieck, 1973) and Crescitelli claims that "the occasional vesicles" that have been reported in the ellipsoids of *F. heteroclitus* do not have the properties of oil droplets. Since then, it has also been found in the cones of the four-eyed fish, *Anableps anableps* (Borwein and Hollenberg, 1973). In both the guppy and the four-eyed fish, the presence of the globular mass or oil droplet could be correlated with a mitochondrial maturation occurring in the ellipsoid of the principal member of the double cone and some single cones. This process culminates in the formation of an oil droplet sensu strictu.
Berger (1966) and Kunz and Wise (1973) described two types of mitochondrial differentiation: one resulting in the formation of oval vesicular-type oil droplets, and the other in the formation of pyriform matrix-type oil droplets. Since the globular mass appears to vary in appearance among the different types of cones of Fundulus, it appeared worthwhile studying its origin with the purpose of examining if it is agrees with either of the cytostructural models proposed by Berger.

B. Material and Methods

The fish employed in this study were kindly provided by Dr. Grace E. Pickford of Hiram College, Hiram, Ohio, USA. There were male killifish, Fundulus heteroclitus brought to Hiram from the New York Aquarium in October, 1971. They were maintained in artificial sea water (Instant Ocean) at a salinity corresponding to that of their natural habitat (Sp. G. 1.0215 at 20°), since Long Island Sound is more dilute than open ocean water. During the winter months they were held at low temperature (4°) and rewarmed in the spring of 1972 to 10° and then 20° as required for the experiments. On account of the sensitivity of hypophysectomised killifish to long daylengths (Pickford, unpublished), they were kept on an 8 h light/16 h dark regime, using standard aquarium reflectors with 15 watt "Grolux" lamps set about 8 inches above the surface of the water. Daily feeding with a modified Aronson mixture (Pickford, 1953) was supplemented twice weekly with frozen brine shrimp.

The fish were decapitated in light or in darkness and the eyes removed and fixed in 3 % glutaraldehyde in sodium cacodylate buffer, and postfixed in 2 % osmium tetroxide buffered with veronal acetate. Prior to fixation the cornea and lens were removed. Fixation lasted 2 h and postfixation, one hour. The rapid embedding technique of Bencosme and Tsutsumi (1970) was followed. The eyes were dehydrated in graded alcohols and propylene oxide and embedded in Epon 812. Sections of 0.5 to 1 μm were cut for light microscopy, and some sections were stained with Azure II-Methylene blue. Thin sections were cut with glass knives using a LKB Ultratome and stained with uranyl acetate and lead citrate. The sections were placed on uncoated copper grids and examined with a RCA EMU electron microscope.

C. Observations

There are three types of cones in the retina of the killifish: the short single cone, the long single cone, and the unequal double cone (Fig. 1). All of them possess a globular structure located at the scleral end of the ellipsoid. This densely stained globule is very sharply defined in the long single cone and in the principal component of the double cone, but not so contrasted in the accessory member of the double cone. Single cones, especially the short ones, have a granular texture in the ellipsoid which is clearly visible in unstained, thick sections.

Electron micrographs of the cone ellipsoids reveal that there exists a vitreal-to-scleral morphological gradient in the mitochondrial population as noted by Berger (1966) in the principal component of the guppy's double cone. However, in the case of the killifish, three different types of globules and mitochondrial maturation, each of these corresponding to different categories of cones, could be identified.

1. The principal component of the double cone, which is usually slightly shorter than the accessory one, possesses ellipsoidal mitochondria which mature to form sclerally a matrix-type oil droplet as defined by Berger (1966). Vitreally, the mitochondria are normal, with disc-like inner membranes (cristae). More sclerally they are swollen and the mitochondrial matrix is filled with a more or less granular, electron dense material (Fig. 2).