

HELICAL FORM CRISTAE IN THE MITOCHONDRIA OF THE PSEUDOBRANCH OF *CYPRINODON VARIEGATUS* ADAPTED TO CONDITIONS OF CONSTANT LIGHT AND DARKNESS

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A functional relationship between the pseudobranch and the eye in teleosts is implied from early studies in which it had been shown that bilateral removal of the pseudobranch will render fish blind (6), and that removal of the eyes or keeping fish in darkness for long periods of time will result in a reduction in the size of the pseudobranch (5). These observations led us to make a pilot study of adult variegated killifish *Cyprinodon variegatus* under conditions of constant light and darkness for varying periods of time to determine if these conditions would stimulate any morphological changes in the pseudobranchial ultrastructure which would correlate with some functional relationship between the pseudobranch and the eye.

METHODS AND MATERIALS

C. variegatus adults were collected from running tidewater pools on the island of Grand Terre, La. and adapted to sea water for 4 days before light and dark adaptation.

The aquaria were enclosed in specially constructed wooden cases which were either illuminated with a fluorescent lamp or completely sealed from light. Control animals were kept in aquaria exposed to a normal alteration of photoperiod. At the end of 7 and 28 days, three fish from each light condition (constant light, constant darkness) were removed and the spinal cord was immediately severed. A red safelight was used for illumination when handling dark-adapted fish. The pseudobranch was quickly excised with the aid of a dissecting microscope.

The tissues were immersed in cacodylate-buffered 1% osmium tetroxide for 1.5 h, dehydrated in methanol, and embedded in Epon. Thin sections were stained with lead citrate and examined with RCA-3F and Siemens IA electron microscopes.

OBSERVATIONS

Mitochondria were abundant in the pseudobranchial epithelial cells of control fish. They were elongate organelles with numerous platelike foliate

cristae (Fig. 1). The mitochondria had an intimate association with the cisternae of the smooth endoplasmic reticulum, as reported for other teleosts (2, 4).

In the fish kept in darkness for 7 days, occasional mitochondria were encountered which showed angulation of some of the cristae, with other cristae arranged in a wavy pattern suggestive of coiling (Fig. 2). In two groups of fish—those kept in light for 7 days and those kept in darkness for 28 days—approximately 5–15% of the observed mitochondria contained one to several profiles of helical cristae amid the usual foliate cristae (Fig. 3).

In the pseudobranch of fish exposed to constant light for 28 days, a larger proportion (70–90%) of the mitochondria contained helical form cristae. These helical cristae were usually aligned in rows consisting of two to eight profiles of cristae. The rows alternated with a single fold of foliate cristae which traversed most of the width of the mitochondrion (Fig. 4). In cross section, the helical cristae had the appearance of tubules with a wall thickness of 210 Å, an outer diameter of 570 Å, and an inside diameter of approximately 140 Å. The wall consisted of two regions, a dense inner membrane about 90 Å thick and a less dense outer layer approximately 120 Å in thickness. The foliate cristae had an average thickness of 230 Å and consisted of two membranes of 70 Å thickness separated by a space which varied from 30 to 90 Å. When the section plane coincided longitudinally with a row of helical cristae, only the helical form of cristae was observed (Fig. 5). However, when the section plane traversed the mitochondria as in *A–B* of Fig. 4, the helical form cristae were oriented longitudinally and alternated with foliate cristae (Fig. 6).

DISCUSSION

Cristae of a helical form are reported here in the pseudobranchial mitochondria of *C. variegatus* which were adapted to conditions of constant light and darkness for varying periods. The low incidence (5–15%) of helical cristae observed in two groups—7 days of constant light and 28 days of constant darkness—compared with the higher incidence (70–90%) and organizational pattern of helical cristae in the group of fish subjected to continuous light for 28 days may represent a specialization of this organelle suggestive of some functional relationship between the eye and pseu-

dobranch of this teleost. The uniform rearrangement of the cristae as rows of helices between foliate cristae could reflect a specialized metabolic activity different from the normal function of the pseudobranchial mitochondria. We conjecture that the helical form cristae represent a modification of foliate cristae.

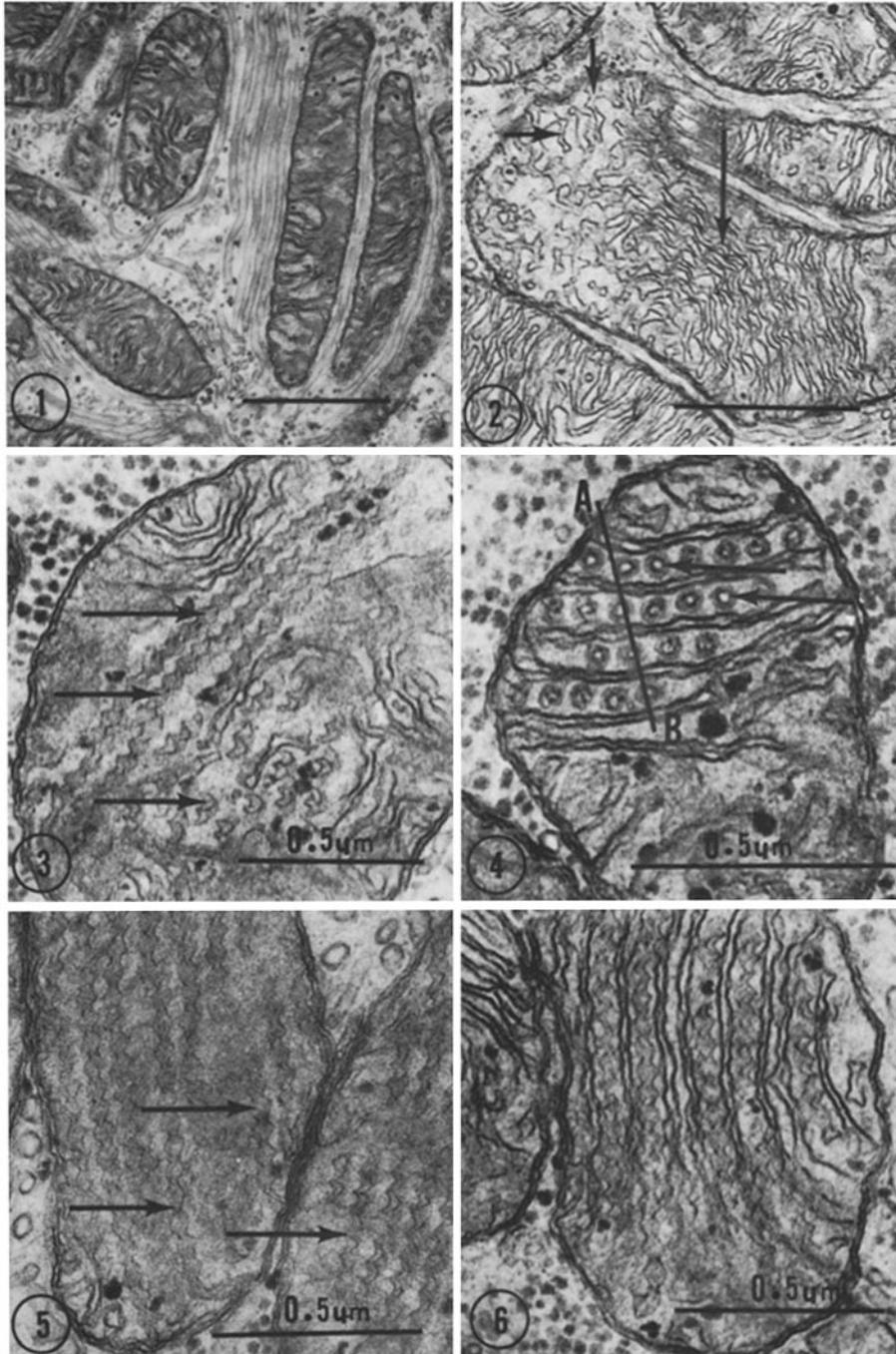
Recent evidence indicates that the pseudobranch may be functionally related to elevated oxygen levels in the eye. Wittenberg and Wittenberg (7) have shown that the ability of marine teleosts to concentrate oxygen in the eye is directly correlated with the relative development of the choroidal gland. Choroidal glands are found almost exclusively in those fish which possess pseudobranchs. Trout have a countercurrent diffusion multiplier behind the retina for the generation of high oxygen tensions (2). Treatment with Diamox, a specific inhibitor of carbonic anhydrase, as well as removal of the pseudobranch will reduce the ocular oxygen tension to the level of arterial blood (2). The pseudobranch contains the highest concentration of carbonic anhydrase of any teleost tissue (4). It is possible that the helical form cristae, as described here, develop as a result of changes in retinal utilization of oxygen during stimulation of constant light and dark conditions which may alter the pseudobranchial output of carbonic anhydrase.

SUMMARY

We have studied, with the aid of the electron microscope, the pseudobranch of *C. variegatus* which had been kept in constant light and darkness for 7 and 28 days, respectively. In two groups (light for 7 days and darkness for 28 days) a small proportion of the mitochondria exhibited helical cristae. In comparison, a larger proportion of the mitochondria in the group kept in constant light for 28 days contained helical form cristae. In this latter group, the helical cristae were aligned in rows which alternated with the usual foliate type of cristae. We interpret this arrangement to indicate a high degree of organization and to suggest a specialized metabolic activity different from the usual function of the mitochondria in the pseudobranch. The helical cristae are interpreted here to be modified forms of foliate cristae.

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