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Architecture of the Ciliary Muscle of *Gallus domesticus*

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ABSTRACT

There are species-related anatomical differences in the ciliary muscle of the avian eye. The arrangement of muscular fibers in the avian eye is not well defined. To clarify this situation, we studied the architecture of ciliary muscle of *Gallus domesticus* by light and scanning electron microscopy (SEM). Our results showed the existence of three main muscular groups that we defined as anterior, posterior, and intermediary. These muscle divisions correspond to the description of the ciliary muscle as previously stated by Crampton (1813), Brücke, and Müller (1856). The striated fibers have a meridian orientation. The anterior and posterior muscular groups are inserted in the sclera, around the Schlemm's canal wall and ciliary process stroma. The vitreal intermediary muscle has fibers inserted in Schlemm's canal wall and ciliary process stroma. The framework of these muscular fibers may according to its insertions participate in the visual accommodation mechanism and outflow of the aqueous humor system.

Key words: ciliary muscle; *Gallus domesticus*; accommodation; maceration and electron microscopy

The ciliary muscle is responsible for the visual accommodation mechanism; through its contractions, it dislocates the ciliary process and alters the tension of zonular fibers. The biconcavity of the lens is modified by this mechanism, allowing the visual field to be focused (Moses, 1988). The existence of a muscle in the ciliary body was suggested by Eustachio in 1560 and described by Brücke and Bowman in 1847 (Duke-Elder and Wybar, 1961). Brücke depicted a group of cells similar to intestine muscle fibers in the anterior portion of the choroid and inserted in the base of the ciliary process and wall of Schlemm's canal. He called this muscle the *tensor chorioidae* muscle. Bowman recognized the presence of these smooth muscle fibers and classified it as a set belonging to the ciliary muscle (Dulke-Elder and Wybar, 1961).

According to Rochon-Duvigneaud (1943), Crampton in 1813 was the first to describe the ciliary muscle in ostrich (*Struthio*) and called it corneal depressor muscle. Afterward, in 1856, the description of similar muscular structures in the eyes of birds was made by Brücke, who described the posterior localization of the choroid tensor muscle in owl (*Bubo orientalis*) and cassowary (*Casuarius*). Also in 1856, Müller studied the falcon (*Accipiter*) eye and described the presence of a posterior muscle that he divided as anterior (Müller's muscle) and posterior

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(Brücke's muscle) groups. These muscular groups represent different functions since they are anatomically separated. In modern anatomical classification, the avian ciliary muscle is formed by Crampton's, Müller's, and Brücke's muscles. The avian ciliary muscle is different from that of mammals because it is striated (Rochon-Duvigneaud, 1943) and structurally similar to skeletal muscle (Sivak and Vrablic, 1982).

The avian ciliary muscle function is still a source of debate. Some suggest it controls the diameter of Schlemm's canal and the angle of the scleral cleft, facilitating the aqueous humor drainage (Evans, 1991; Ying, 1995). The role played by the ciliary muscle in accommodation of the lens and cornea was demonstrated in avian eye (chicken and pigeon) (Evans, 1991). Accordingly, Glasser and Howland (1995) determined that contraction of the ciliary muscle caused a change in the curvature of the cornea for corneal accommodation. The posterior ciliary muscle pulls the posterior ciliary body forward against the tension of the tenacular ligament. It was established that the lens plays a major role in vertebrate accommodation; its optical properties during accommodation have been difficult to assess partly because the lens was located within the eye (Choh et al., 2002).

The description of the ciliary muscle in the chicken is also open to debate. Nickel et al. (1977) described the ciliary muscle as divided into two or three muscular groups: one external and composed of Crampton's muscle, which were connected to the tissue that covers the scleral ring bone; a second muscular group composed of Brücke's muscle coupled to the sclera; and a third muscular group composed of Müller's muscle that appears as a small segment of Brücke's muscle. In contrast, Suburo and Marcantori (1983) studied the muscles involved in eye accommodation and suggested that the ciliary muscle was composed of only two muscular groups, one distal and another proximal, represented by Crampton's and Brücke's muscles, respectively. The aim of this work was to describe by ultrastructural analysis the architecture of the ciliary muscle in chicken's eyes and define the muscular groups in detail.

MATERIALS AND METHODS

Animals and Sample

Thirty adult white leghorn chickens (*Gallus domesticus*) were used. Animals were obtained in commercial aviaries and killed by decapitation according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996). Eyes were enucleated, fixed as described below, and sectioned dividing the bulb in two segments (anterior and posterior). However, only the anterior segment was analyzed. The anterior segment was divided in two portions, one processed for light microscopy using historesin and the other for scanning electron microscopy (SEM).

Historesin Preparation

Samples were immersion-fixed for 12 hr at 4°C with 2.5% glutaraldehyde diluted in 0.1 M Na-cacodylate buffer, pH 7.4. After fixation, the samples were immersed in 5% EDTA solution for 7 days for decalcification. The material was dehydrated in a graded ethanol series and embedded for 12 hr in 100% ethanol and historesin [glycol metacrilate (GMA); Leica; 1:1] under slow stirring. Blocks were cut at a thickness of 2 μm (American Optical 820) and stained with 0.1% toluidine blue in 1% sodium borate. Slides were mounted in entellan (Merck, Damstadt, Germany) and observed in a Nikon Optiphot-2 microscope.

Scanning Electron Microscopy

The anterior segment samples were fixed as described above, washed in 0.1 M Na-cacodylate buffer, pH 7.4, and postfixed for 1 hr with 1% osmium tetroxide in 0.1 M Na-cacodylate buffer, pH 7.4. After washing in the same buffer, the material was dehydrated in a graded ethanol series, fragmented in radial sections, dried by CO_2 critical point (Balzers, CPD 030), mounted in appropriated support with colloidal silver print, and coated with gold (Sputtering, Balzers, SCD 050). Samples were analyzed under scanning electron microscope (JEOL, JSM 5300).

Maceration Methods

After fixation, the anterior eye segments were cut into small pieces and immersed in 1% osmium tetroxide in 0.1 M Na-cacodylate buffer, pH 7.4 (maceration solution). Maceration was performed according to the microwave irradiation method as described by Tanaka and Naguro (1981) and Hotta et al. (1990). The fragments were deposited in a beaker with 25 ml maceration solution inside an ice bath and submitted to intermittent 1-sec microwave irradiation for 60 min with a maximum temperature of 40°C. We also used slow maceration at 4°C for 15 days (Nakamura and Yamamoto, 1988). The samples were processed for SEM as described above.

RESULTS

The ciliary muscle of *Gallus domesticus* is located in the anterior eye segment and is inserted in the sclera, near Schlemm's canal wall, and separated from the ciliary process by the ciliary cleft (Fig. 1A and B).

Light microscopy showed that the ciliary muscle was composed by striated fibers with alternating dark and light bands transversally disposed along the fiber axis (Fig. 1C, arrowheads). By SEM, after maceration, the transverse striations of the muscular fibers were more clearly seen (Fig. 1D, arrowheads). These fibers were present in the stroma of the ciliary body, between the sclera and the ciliary cleft. Thus, the ciliary muscle was composed of three muscular groups defined by its insertions and anatomical position in the eye as anterior (Crampton's muscle), intermediary (Müller's muscle), and posterior (Brücke's muscle; Fig. 2A and B). The muscle fibers cross all portions of the ciliary muscle, mainly in a radial manner.

Anterior Muscular Group (Crampton's Muscle)

The fibers of the ciliary muscle have variable lengths. The fiber set present in the anterior muscular group of the ciliary muscle is a major component of the muscle. The anterior insertion occurs in the tissue around Schlemm's canal (Fig. 2C and D) and is formed by sclerocorneal transition tissue. The posterior insertion is localized in the scleral conjunctive tissue, near the scleral bones (Fig. 2E). In this muscular group, the fibers form a small tendon that is intermixed in the scleral tissue and forms a sharp angle with the sclera (Fig. 2E, arrow).

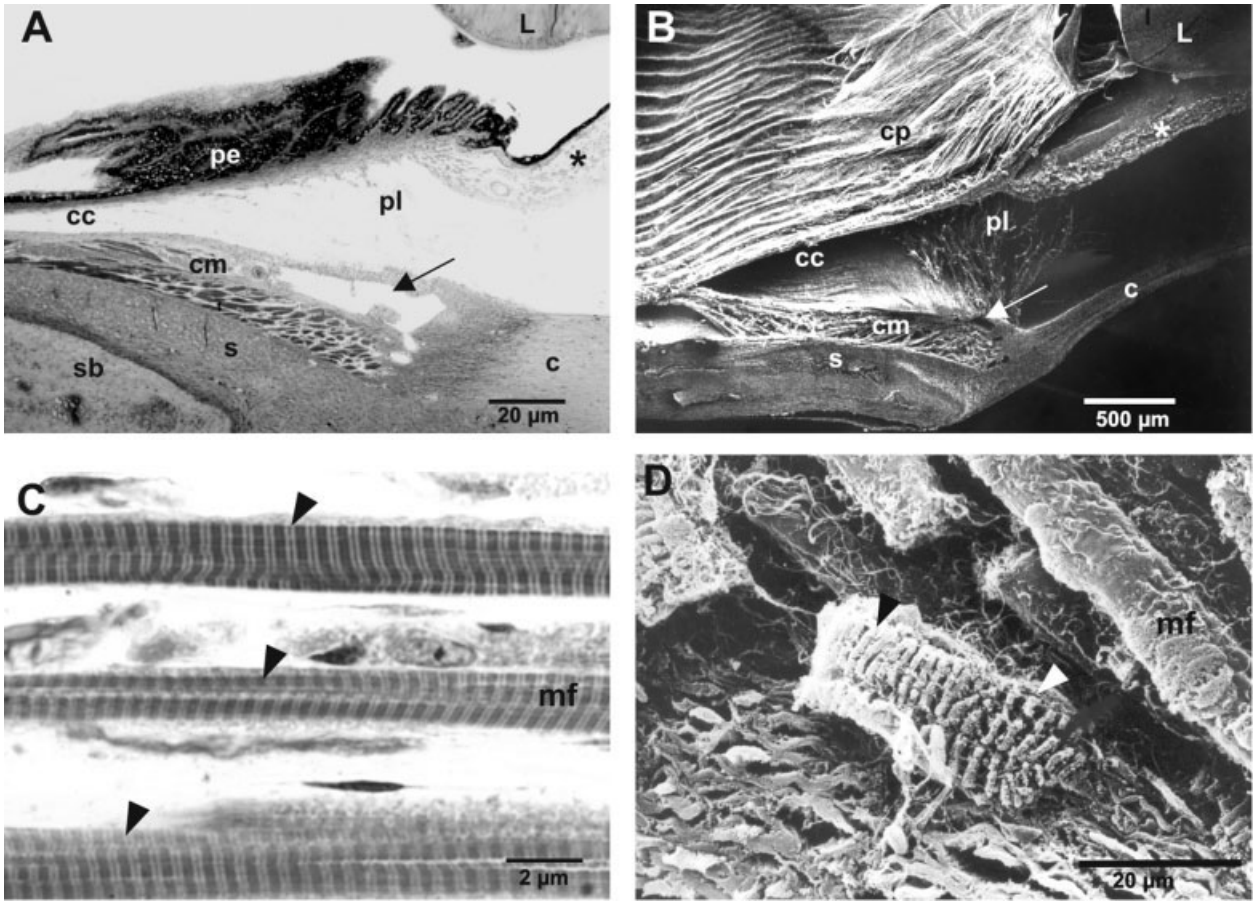

Fig. 1. Light microscopy (**A**) and scanning electron micrograph (**B**) of the meridional section of the eye show the anatomical localization of the ciliary muscle (cm) and the internal structures of this segment: lens (L), iris (*), ciliary process (cp), pectinated ligament (pl), ciliary cleft (cc), cornea (c), sclera (s), scleral bone (sb), and Schlemm's canal (arrow). Using light microscopy and sections stained with toluidine blue (**C**) and maceration technique followed by SEM (**D**), striated (arrowhead) muscle fibers (mf) were observed.

Intermediate Muscular Group (Müller's Muscle)

This muscle was located nearer to the vitreal portion of the eye than to other regions of the ciliary muscle. Its fibers have a radial disposition beneath the endothelium of the ciliary cleft and cross the ciliary cleft (Fig. 2A). The anterior insertion was around the wall of Schlemm's canal, intermingled with the fibers of the anterior muscle. The posterior insertion occurred in the stroma of the ciliary process and choroid (Fig. 2A and E).

Posterior Muscular Groups (Brücke's Muscle)

The anterior insertion (scleral) occurred in the scleral conjunctive tissue, near the scleral bones located in a posterior position with regards to the anterior muscular group of the ciliary muscle. The posterior insertion connected with the intermediate muscular group of the ciliary stroma and choroid through fine tendons (Fig. 2F and G).

The ciliary muscle fibers form an acute angle with the sclera and an obtuse angle within each other (Fig. 2A, B, and G). The anterior insertions occupied a larger area than the posterior insertion, conferring a fan aspect to the muscle group as observed in SEM. The anterior and posterior muscular groups of the ciliary muscle were inserted into the sclera and this defined the anatomical limits of the muscle (Fig. 2A and B).

DISCUSSION

Early studies of muscle fibers were performed by dissection and dissociation. Muscle fibers in birds were striated and initially described in the 19th century by Crampton, Brücke, and Müller (Rochon-Duvigneaud, 1943). Afterward, the structure of the ciliary muscle was defined by histological techniques.

The architecture of the ciliary muscle in man was defined by dissection and dissociation under a stereoscopic microscope (Calazans, 1953). Using electronic microscopy, Ishikawa (1962) confirmed that fibers of the human ciliary muscle were similar to visceral smooth muscular fibers. However, in birds' eyes ciliary muscle fibers were composed of striated fibers similar to those found in skeletal muscle (Sivak and Vrablic, 1982). We have confirmed these findings.

Tissue digestion (Ohtani, 1987; Nakamura and Yamamoto, 1988) and maceration associated with microwave irradiation (Tanaka and Naguro, 1981; Hotta et al., 1990) allowed us to study the structure of the ciliary

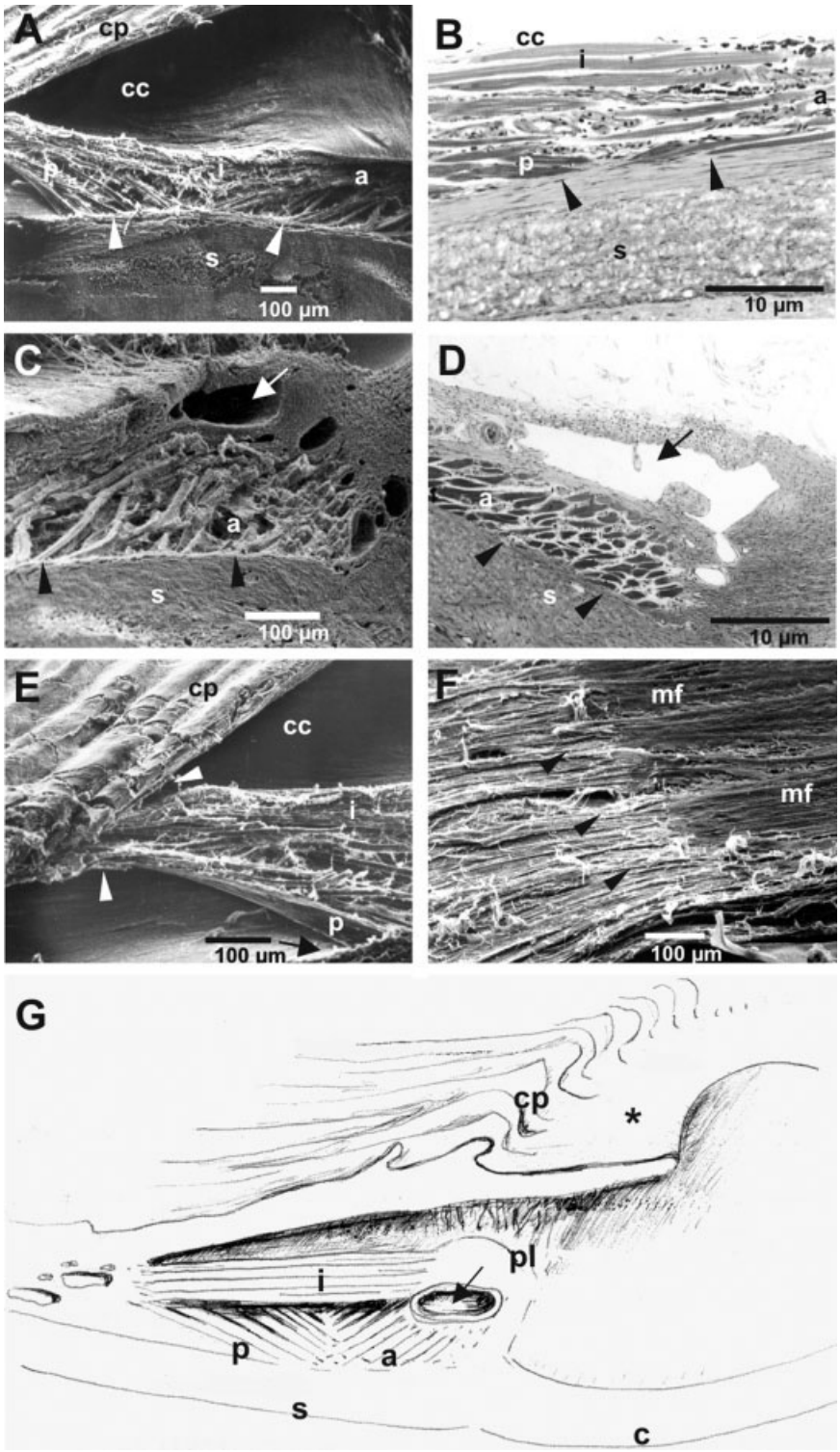

Fig. 2. In **A** and **B**, the three groups of the ciliary muscle and its insertions (white arrowheads and black arrowheads) were observed. In **C** and **D**, a detail of the anterior insertion (scleral; arrowhead) of the anterior muscular group and its relationship with the Schlemm's canal (arrow) were observed. **E:** Insertions of posterior muscular group (p) in the sclera (arrow) and the posterior insertion of the intermediate muscular group (i), both in the stroma of ciliary process (arrowhead). **F:** The fine tendons of the muscular fibers inserted in the stroma of ciliary process were observed after dissection (arrowhead). **G** is an illustration that demonstrates the structure that from the anterior segment of the ocular bulb: sclera (s), cornea (c), ciliary process (cp), iris (*), pectinated ligament (pl), ciliary muscle (cm), anterior (a), intermediate (i), and posterior (p) muscular group and Schlemm's canal (arrow).

muscle by SEM, while previous studies were based on histological sections and dissections documented by drawings. Previous studies using SEM of the ciliary muscle in birds (Suburo and Marcantoni, 1983) and nonhuman primates (Nishida, 1986) used chemical digestion of the tissues, which in our hands produced poor resolution when applied to the ciliary muscle architecture. Even the chemical digestion method described by Tanaka and Naguro (1981), which used HCl or NaOH, did not produce sufficient exposure of the ciliary muscle in the absence of other manipulations. Therefore, microdissecation to remove the ciliary process was necessary.

Suburo and Marcantoni (1983) performed their SEM studies of the ciliary muscle by digestion with hyaluronidase. They observed two muscles: the anterior (Crampton) and the posterior (Brücke), inserted in the scleral wall of Schlemm's canal and in the ciliary body *pars plana*. The authors concluded that, in *Gallus domesticus*, there was no third intermediate muscle (Müller's muscle). The technique used by the authors was probably not adequate to observe Müller's muscle. Despite clear preparations, Nishida (1986) did not elucidate the architecture of the ciliary muscle in detail and did not define any specific anatomical section.

Murphy et al. (1995) used light microscopy and methacrylate embedding and observed two muscular groups (anterior and posterior) with a fusiform musculature that belonged to a subdivision of the anterior group they termed the intermediate musculature. In the present article, we showed that the fusiform musculature described by Murphy et al. (1995) was an independent muscle denominated by us as the intermediate muscular group and not a subdivision of the anterior muscular group. The use of light microscopy and not SEM (Murphy et al., 1995) did not allow the authors to observe the three-dimensional disposition of the ciliary muscle and its insertion.

In the present investigation, we described the ciliary muscle architecture as formed by three muscular groups: anterior, posterior, and intermediate. The intermediate muscular group refers to the muscular fibers that correspond to Müller's muscle (*fibrae radials*). The existence of this muscle as an individual portion of the ciliary muscle has been questioned in the chicken eye. In the Avium Anatomical Nomina (Evans, 1991), the disposition of the fibers in the ciliary muscle was not considered as individualized, but if functional analysis was required, they would have to be studied separately.

The architecture of the avian ciliary muscle was also open to discussion because it presented genus- and species-specific differences. Evans (1991) considers that the ciliary muscle in birds has two to four subdivisions while Duke-Elder (1958) described the ciliary muscle as divided in two portions (Brücke and Müller's muscle). Nickel et al. (1977) suggested two main portions: external (Crampton's muscle) and posterior (Brücke's muscle), as well as a small segment inside the posterior muscle (Müller's muscle). In the chicken, Suburo and Marcantoni (1983) described a ciliary muscle formed by two portions (anterior and posterior).

The differences in avian ciliary muscle were directly associated with habits and environment. Thus, nocturne predators (owl, *Strigidae*) have a well-developed Crampton's muscle, while in aquatic birds this muscle was less developed or absent (Walls, 1942; Duke-Elder, 1958). In contrast, the pigeon had a transversal muscle in this portion whose nomenclature was not yet defined. Another controversial situation was present in the ciliary muscle of the cormorant (Duke-Elder, 1958).

The ciliary muscle has been described as a structure belonging to the ciliary body since its localization was predominantly scleral except for the posterior insertion in the projection of the ciliary process in the choroid and stroma. This situation fits well in birds, where the ciliary muscle is inserted in the sclera, separated from the proper ciliary body by the ciliary cleft, a space full of aqueous humor in continuity with the anterior chamber through the pectinate ligament. However, in mammals the ciliary muscle architecture was more complex, with smooth fibers crossing in various directions (Calazans, 1953).

Knowing the disposition and insertions of the ciliary muscle in the eye of *Gallus domesticus* allows us to suggest a functional interpretation. Thus, the posterior and intermediate muscular groups project to the ciliary process in the inner and anterior direction, acting on the ciliary zonule and lens and altering optic properties (Nickel et al., 1977). In this case, the posterior insertion presents more mobility.

It is well known that the image focalization mechanism in *Gallus domesticus* is different from mammals. According to Evans (1991), the bulb functions as a hydrostatic unity, in which, the alterations in lens position and shape, and corneal curvature are provoked by changes in the intraocular pressure forcing the center of lens into a constricted aperture in the iris.

In addition, the anterior portion (Crampton's muscle) has an anterior insertion near the corneal stroma in the limbus portion, which can modify the corneal curvature during accommodation. On the other side, the posterior insertion in the sclera was fixed near the scleral bones. This allowed the corneal radius curvature to change during accommodation while stabilizing the remaining structure (Duke-Elder, 1958; Nickel et al., 1977; Troilo and Wallman, 1987).

There are many descriptions of lenticular accommodation in bird eyes. It has been proposed that alteration in lens curvature was caused by contractions of the ciliary muscle or by contractions of both muscle groups together. Glasser et al. (1995) concluded that the iris was the principal structure responsible for enlargement of the lens power during accommodation.

Moreover, the insertion of the anterior fibers of the ciliary muscle in the sclera around the wall of Schlemm's canal suggested a possible functional relationship with the drainage system of the aqueous humor via the ciliary cleft (Ying, 1995). In conclusion, our results showed clearly the existence of three muscular groups in the ciliary muscle of *Gallus domesticus*.

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