Functional Anatomy of the Ciliary Muscle in Birds and Humans

by

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Abstract

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The functional anatomy of the avian ciliary muscle has been difficult to describe due to its inaccessible location inside the eye. This thesis compares the ciliary muscle of birds and humans in both the relaxed and contracted states. The two groups both accommodate by changing the shape of the lens, although the ciliary body and ciliary muscle morphology are very different.

The ciliary muscles of the chicken (*Gallus gallus domesticus*), the pigeon (*Columbia livia*), the kestrel (*Falco sparverius*), and the hooded merganser (*Margus cucullatus*) (four species with differing accommodative needs) were examined histologically. The ciliary muscle of all four species can be divided into three main muscle fibre groups based on insertion and origin: anterior, posterior and internal. The anterior muscle fibre group originates at the sclera under the scleral ossicles and inserts into the inner lamellae of the cornea. During accommodation these fibres pull the cornea posteriorly, changing the curvature of the cornea. The posterior muscle fibre group originates on the sclera and inserts posteriorly onto the baseplate of the ciliary body. The posterior fibre group acts on the baseplate of the ciliary body, pulling it forward to change the curvature of the lens during accommodation. The internal muscle fibre group extends from the baseplate of the ciliary body to the inner lamellae of the cornea and thus has a role in both corneal and lenticular accommodation. Species differences do exist as seen in subgroups determined by the orientation of the fibres

between the relaxed and contracted states and the percentage of fibres within the main muscle fibre groups. In general, the majority of ciliary muscle fibres in the chicken, pigeon, and kestrel are in the anterior muscle fibre group, suggesting an emphasis on corneal accommodation; in the ciliary muscle of the hooded merganser, the majority of fibres are in the internal and posterior muscle fibre groups, indicating that lenticular accommodation is the predominant form of accommodation.

For the study of human ciliary muscle, fifteen pairs of eyes ranging in age from 0 to 107 years were treated with 20% pilocarpine and 5% atropine. The ciliary muscle decreases in length (8-20%) and in width (5-14%). Changes with contraction were not found in the other measurements. The fibres of the ciliary muscle do not change orientation with contraction. During accommodation the ciliary muscle releases zonular tension by shortening, but not by moving forward. With age the ciliary muscle shortens, widens, and moves forward. Changes in ciliary muscle dimensions were seen at all ages, indicating no loss of muscle contractility. The amount of connective tissue in the ciliary muscle increases (24%) with age. The aging ciliary muscle is able to contract, but the reduction of zonular tension brought on by age, may prevent lenticular changes, thereby contributing to presbyopia.

Acknowledgments

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General Introduction

The role of the ciliary muscle in accommodation is difficult to describe due to its location within the eye. However, its importance in initiating the changes in lens shape associated with accommodation is not disputed. The objective of this thesis is to examine the role of the ciliary muscle in accommodation by studying the anatomical connections of the muscle and examining the changes in the muscle between the relaxed and contracted state. This is accomplished by examining the ciliary muscle in two animal groups: birds and humans. Birds and humans accommodate through a change in lens shape, although ciliary body and ciliary muscle morphology are considerably different. The thesis examines the similarities and differences in ciliary muscle structure and function between these two groups and analyzes how ciliary muscles that are anatomically very different can achieve a lenticular refractive change. This approach is the first systematic, morphological comparison of the ciliary muscle of birds and humans in which ciliary muscles have been pharamologically treated to induce contraction and relaxation. The following section is an overview of the vertebrate eye and its accommodative mechanisms. The anatomy of the ciliary body in humans and birds will then be described, followed by a description of accommodation in these two groups. The general anatomy of muscle will be described, followed by a closer examination of the ciliary muscle of birds and humans. The last section of the General Introduction will outline the specific goals and organization of the thesis.

The Vertebrate Eye

The typical vertebrate eye consists of a collagenous shell with a transparent window, an optical lens, a muscle to change the focus of the eye and a sensory retina (Walls, 1942). The scleral portion of the collagenous shell consists of disorganized collagen fibres of various diameters (Hogan *et al.*, 1971). The transparent window, the cornea, is created when collagen fibres of uniform size become organized into lamellae in which collagen fibres are arranged at a uniform distance from each other (Maurice, 1957). The collagen fibres in the lamellae all run parallel to the surface of the cornea and each lamella is arranged at right angles to adjacent lamellae. The ocular lens is formed by crystalline fibres arranged in concentric rings around an older nucleus (Hogan *et al.*, 1971). In the mammalian eye, the ciliary body surrounds the lens, suspending it in the eye along the visual axis. The ciliary body mainly consists of the ciliary processes and the ciliary muscle, which directly or indirectly change the shape of the lens and/or cornea. All of the preceding structures direct light onto the retina where visual information is processed.

In order for an image to be focused on the retina, incident light rays are refracted to form an image on the retina. The cornea, aqueous humour, lens, and vitreous humour all play a refractive role, although the cornea is most important (Helmholtz, 1909). For the eye to be focused on either a near or far object, the power of the eye has to change to enable the light rays to remain focused on the retina. This process of changing the focus of the eye is called accommodation. The vertebrate eye accommodates in various ways: by changing the shape of the lens (birds, reptiles, and primates), the position of the lens

(fish), and/or the shape of the cornea (birds) (Walls, 1942; Sivak, 1980). [See Gillum, 1976; Sivak, 1980; Munk, 1996 for reviews.] Variation in the position of the retina [ramp retinas in stingrays (Sivak, 1980); choroidal thickness changes in chicks (Wallman *et al.*, 1995)] or changes in the refractive index of the lens may also allow for accommodation, although the latter method does not exist in any known animal.

The following work concentrates on organisms that change the shape of their lens during accommodation, specifically, humans and four avian species.

Ciliary Body Anatomy

Humans

The ciliary body is a circular structure that extends from the scleral spur to the ora serrata. It has a triangular shape with the base of the triangle facing the anterior chamber and the apex at the ora serrata (Hogan *et al.*, 1971)(fig. 1). The length of the ciliary body, from anterior to posterior points, is shorter nasally (4.5 to 5.2mm) and longest temporally (5.6 to 6.3 mm) (Hogan *et al.*, 1971). The ciliary body is divided into two parts: pars plicata and pars plana (Hogan *et al.*, 1971). The pars plicata contains the ciliary processes, while the pars plana extends from the posterior end of the ciliary muscle to the ora serrata, where the retina begins. The human ciliary body is composed of 1) ciliary muscle, 2) layer of vessels and ciliary processes, 3) epithelium, and 4) stroma.

The human ciliary body is an embryological continuation of the structures of the posterior portion of the eye (Snell and Lemp, 1989). The choroid is continuous

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Figure 1: A schematic drawing of the human ciliary body showing the indirect

attachment of the ciliary processes (P) to the lens via the zonules (Z). The three fibre

orientations of the ciliary muscle are visible: longitudinal (L), radial (R), and circular

(C). The tension fibers (T) attach the main fibre system to the ciliary body and course

both anteriorly and posteriorly. (I): iris.
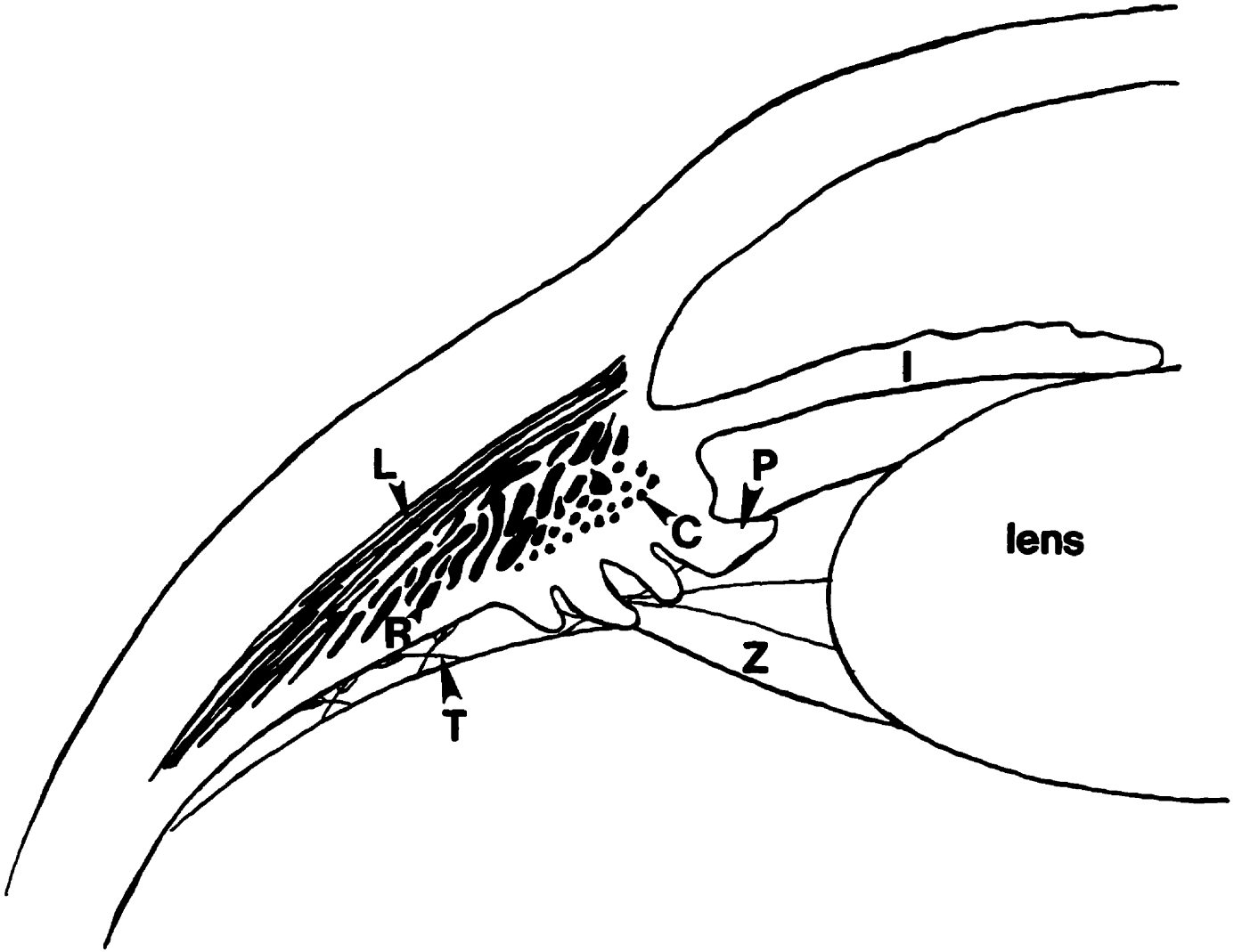
anteriorly as the ciliary muscle while the sensory retina becomes the ciliary

unpigmented epithelium and the retinal pigmented epithelium becomes the ciliary

pigmented epithelium. The ciliary muscle is generally described as a smooth muscle

(see General Muscle Structure below) with three muscle fibre orientations: circular,

radial and longitudinal (fig. 1). There are approximately 70 to 80 ciliary processes

around the eye (Duke-Elder, 1961). These processes contain a large capillary bed and

are covered by the pigmented and unpigmented epithelial layers. The unpigmented

epithelium is the innermost layer of the ciliary body while the pigmented epithleium is a

single layer that lies external to the unpigmented layer in the eye, covering the ciliary

body from the root of the iris to the ora serrata. The ciliary processes form a ring around

the lens but do not make contact with it (fig. 1). The stroma of the ciliary body contains

fibroblast and pigment cells, collagen fibres, blood vessels, and nerves (Hogan *et al.*,

1971). An inner layer of connective tissue is thick in the pars plicata and thin in the pars

plana. The ciliary muscle is mainly innervated by the short ciliary nerves which branch

off the oculomotor nerve and are of parasympathetic origin (Hogan *et al.*, 1971).

Innervation from the sympathetic system is via the first division of the trigeminal nerve

(Kaufman, 1992).

The human crystalline lens is suspended from the ciliary body by the zonules of

Zinn (Hogan *et al.*, 1971) which extend from the lens capsule to the valleys between the

ciliary processes, along the pars plicata and pars plana (Farnsworth and Burke, 1977).

The zonules attach to the lens at three points: the equator, the anterior peripheral edge

and the posterior peripheral edge (fig. 1). They originate at the unpigmented epithelium

of the ciliary body (Hogan *et al.*, 1971).

Birds

The anatomy of the avian ciliary body is very different from that found in humans. The ciliary muscle of birds is not continous with the epithelial layers as in humans. In avian species, the ciliary body is divided by a ciliary cleft which results in an outer leaf containing the ciliary muscle, tracebular meshwork, and aqueous sinus and an inner leaf composed of the baseplate of the ciliary body (Tripathi, 1974) (fig. 2). The ciliary processes and epithelial layers are attached to the internal surface of the baseplate. The avian ciliary muscle is a striated muscle (see below), although innervated by the autonomic nervous system (Walls, 1942). Historically, it has been divided into three muscle groups: Crampton's muscle, Brücke's muscle and Müller's muscle (Meyer, 1977)(fig. 2). More recently, the literature has become confused on the location of these groups and their location and/or existence in different species. This issue will be addressed in Section I. The avian ciliary muscle inserts directly into the lamellae of the cornea (Glasser *et al.*, 1994), unlike the human ciliary muscle which inserts onto the scleral spur. The direct attachment of the ciliary muscle onto the cornea allows certain birds to accommodate by changing corneal shape as well as that of the lens. The avian lens is surrounded by an annular pad formed by the radial thickening of equatorial epithelial cells (Willekens and Vrensen, 1985) (fig. 2). The annular pad bridges the space between the lens and ciliary processes so that the ciliary processes make direct contact with the lens. The bird eye also contains scleral ossicles: a ring of overlapping bones in the sclera positioned over the ciliary body (fig. 2). The scleral ossicles are responsible for the formation of the corneo-scleral sulcus (Walls, 1942),

Figure 2: A schematic drawing of a typical avian ciliary body. The ciliary processes

(CP) attach directly onto the lens (L) via the baseplate of the ciliary body (B). The three

fibre orientations within the ciliary muscle are distinguishable: the anterior fibres (A),

the posterior fibres (P) and the internal fibres (I). The scleral ossicles (O) create a

corneo-sceral sulcus which brings the ciliary body closer to the lens. (IR): iris, (AP):

annular pad.
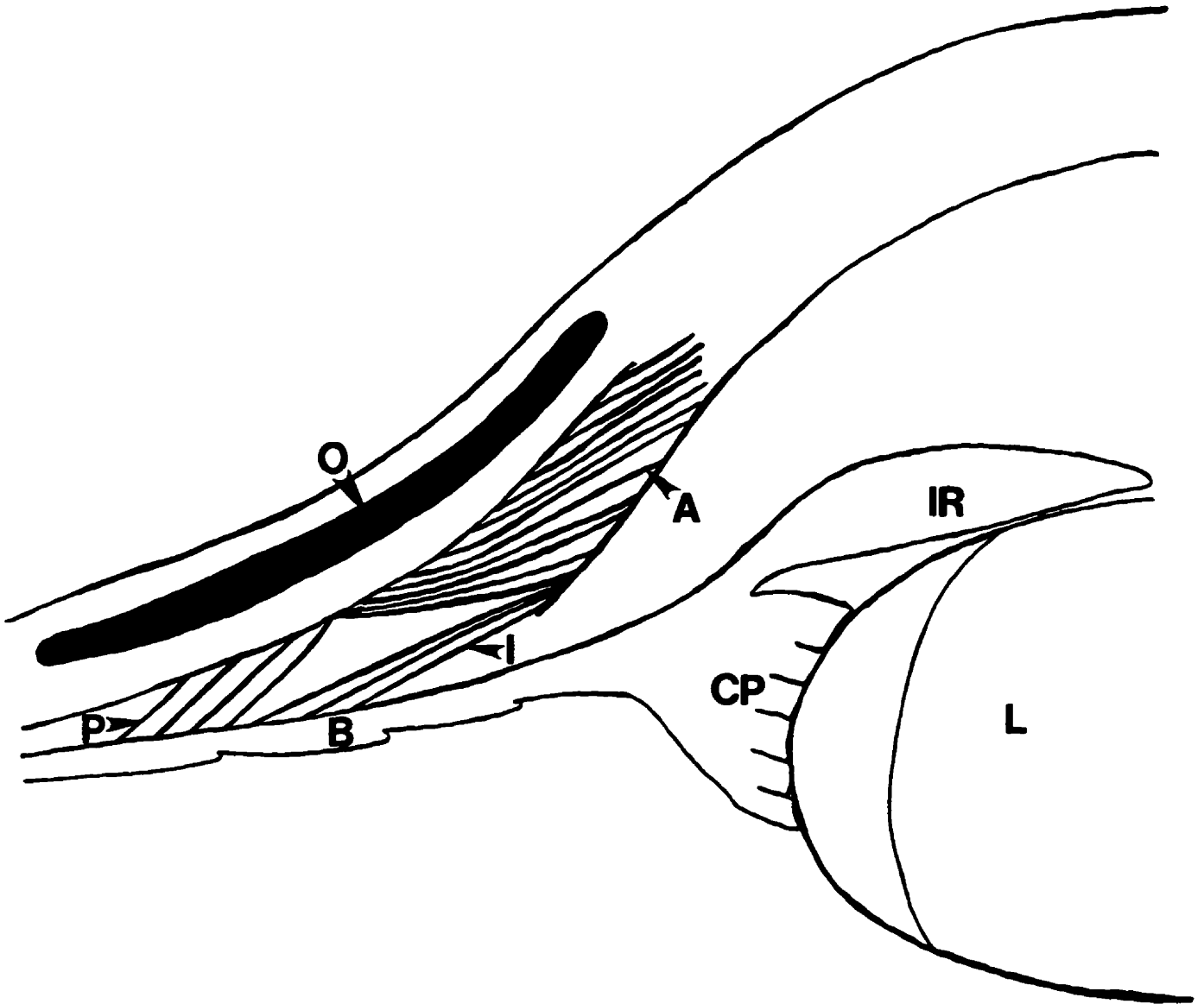
another feature of the eye responsible for the direct contact between the lens and ciliary body.

General Muscle Structure

The three general classifications of all muscle are: a) striated, b) smooth, and c) cardiac. Striated or skeletal muscle consists of elongated, multi-nucleate muscle fibres (Briggs, 1991). Each muscle fibre contains numerous myofibrils composed of myosin and actin filaments. The myosin filaments lie between the actin filaments, with the ends of the filaments overlapping. A myosin segment with its corresponding actin ends is referred to as a sarcomere. The arrangement of the myofilaments in the sarcomere allows the two types of filaments to slide together during muscle contraction. The ordered arrangement of these contractile filaments form light and dark bands (the dark band representing the thicker, myosin filaments) in the striated muscle fibre. Striated muscle is innervated by the somato-motor system and has a fast innervation response time (Briggs, 1991).

Smooth muscle consists of smaller fibres with a single nucleus located near the middle of the fibre (Ham and Cormack, 1979). The thick and thin filaments are not arranged in sarcomeres and this results in an unstriated, smooth appearance. The cells contain few mitochondria or intracellular organelles and the sarcoplasmic reticulum is less developed compared to other muscle cell types. These muscle fibres form bundles surrounded by connective tissue, with the ends of bundles interdigitating with other bundles. This arrangement creates a group of bundles that functions as a single unit

(syncytium). While smooth muscle is not divided into sarcomeres, the fibres do contain dense bodies and intermediate bodies. The intermediate bodies pull on the dense bodies which are scattered throughout the fibres. The portions of the cell between the dense bodies balloon out during contraction and the nuclei become pleated. It is not exactly clear how myosin and actin play a role in smooth muscle contraction, but they are thought to have a sliding filament mechanism, as in striated muscle. Smooth muscle is innervated by the autonomic nervous system and is characterized by the relatively low response level and continuous contraction. There are two types of smooth muscle: visceral smooth muscle and multi-unit smooth muscle. In visceral smooth muscle, few fibres in the bundle have neuromuscular junctions. In multi-unit smooth muscle every fibre is innervated individually. This results in fast localized contraction, more characteristic of striated muscle fibres (Ham and Cormack, 1979).

The third type of muscle, cardiac muscle, is found exclusively in the heart and has both striated and smooth muscle characteristics. The muscle is striated in appearance and has a well developed sarcoplasmic reticulum. Cardiac muscle, however, is unique because cardiac cells are joined by intercalated discs which rapidly spread a contraction through the heart muscle. The muscle fibres contain one or two nuclei and a large number of mitochondria to sustain its continuous activity. Cardiac muscle is under autonomic control (Ham and Cormack, 1979).

Ciliary Muscle Structure

Humans

The ciliary muscle and the iris muscles are unusual for they are classified as smooth muscles in mammals and striated muscles in birds and reptiles. The mammalian ciliary muscle is often described as smooth muscle, due to its unstriated appearance and autonomic innervation. A comparative study of the ciliary muscle of various mammals and that of chickens revealed that the mammalian ciliary muscle contains basophilic granules in species with greater accommodative amplitudes (Woolf, 1956). This granular appearance differs from other smooth muscle (i.e. visceral). Woolf compared the granular appearance of the mammalian ciliary muscle to the appearance of the embryonic chick ciliary muscle, to determine if there was some similarity in granular appearance to striated muscle. No similarities were found between chicken ciliary muscle anatomy and that found in mammals. Ishikawa (1962) has shown the human ciliary muscle to be an atypical smooth muscle. Like other smooth muscle, the human ciliary muscle has a single nucleus per fibre that is located centrally, with the axis of the nuclei parallel to the longitudinal axis of the cell. However, unlike other smooth muscle, the cells of the human ciliary muscle contain a well-developed Golgi apparatus and an abundance of mitochondria (Ishikawa, 1962; Van der Zypen, 1967). There is also a systematic arrangement of endoplasmic reticulum which resembles the sarcoplasmic reticulum framework of striated muscles (Ishikawa, 1962). Ishikawa reported that the myofilaments are distributed throughout the fibres and are arranged longitudinally. He also reported the existence of many nerve fibres, postulating that

each cell may have its own innervation. The fibres are packed in bundles with a cellular sheath around the fibres. Adjacent fibres appear to touch. Ishikawa suggests that this is similar to the intercalated discs in cardiac muscle and that these attachment sites are possible pathways of conduction for muscle contraction. Gap junctions have not been found between the muscle cells (Tamm and Lütjen-Drecoll, 1996). The ciliary muscle cells are reported to contain dense bands that have been compared to Z-bands in striated muscle (Tamm and Lütjen-Drecoll, 1996).

The human ciliary muscle, as described above, fits the description of a multi-unit smooth muscle: each fibre is innervated and the fibres are arranged in bundles which join in branches. The human ciliary muscle exhibits a faster response time than typical smooth muscle, as expected from the abundance of mitochondria and the rich innervation (Ishikawa, 1962; Van der Zypen, 1967). The primate ciliary muscle has also been classified as a fast multi-unit smooth muscle by Bozler (1948) (Tamm and Lütjen-Drecoll, 1996).

Birds

The avian ciliary muscle is a striated muscle. There exists very little data on the ultrastructure of the avian ciliary muscle. One study examined the ultrastructure of intraocular muscles of diving and nondiving ducks (Sivak and Vrablic, 1982). This study found the ciliary muscle of both species to have multinucleate muscle fibres and abundant mitochondria within the muscle cells. The diving duck muscle cells contain a more highly developed transverse tubule system and the neuromuscular junctions consist of diffuse multiple endings whereas in the domestic duck, the cells contain

diffuse single end plate neuromuscular endings. The added neuromuscular junctions may provide more stimulation to the ciliary muscle of the diving duck to squeeze the lens to a greater degree. A diving duck needs more accommodative power underwater due to the neutralization of corneal power by the aqueous humour and water (see Section I).

Accommodation

Humans

An examination of the historical theories of human accommodation reveals three general hypotheses on how the shape of the lens changes with ciliary muscle contraction: a) due to an increase in zonular tension, b) due to a decrease in zonular tension, or c) due to the action of the choroid, vitreous, and iris. These theories are not necessarily exclusive of each other. A primary theory of accommodation, first proposed by Helmholtz in 1909, states that the ciliary muscle contracts for near vision, releasing the tension on the zonules. This reduces the tension on the pliable lens and allows the lens to become thicker, thereby increasing its refractive power. For far vision, the ciliary muscle relaxes, pulling the zonules taunt which in turn flattens the lens. In 1895 Tscherning theorized that the lens did not increase in thickness due to a release of zonular tension, as proposed by Helmholtz. Rather, the lens flattens peripherally, with a central steepening during accommodation. Tscherning claimed that zonular tension increased with muscle contraction, causing peripheral flattening of the lens. As the

ciliary muscle contracts, the choroid is pulled forward, forcing the vitreous against the lens and holding it in place (Tscherning, 1920). Fincham (1937) agreed with the basic theory of accommodation proposed by Helmholtz and provided additional evidence for lens thickening. However, Fincham did not believe that the lens itself was elastic and would rebound to a rounder shape when zonular tension was released. His experiments led him to propose that the lens substance was "soft" while the capsule was the elastic force shaping the accommodated lens (Fincham, 1937). Therefore, the capsule would be producing the central steepening and peripheral flattening of the lens.

While Helmholtz's theory of accommodation has been the mostly widely accepted, the complexity of accommodation is demonstrated by ongoing debates in the literature on the mechanism of accommodation. Many of the "new" theories are based on revived theories from the past.

Coleman (1970, 1986) has proposed a hydraulic theory of accommodation based on changes in intraocular pressure, the support of the lens by vitreous, and the pull on the choroid by the ciliary muscle during contraction. Coleman's theory combines parts of the accommodative theories of Helmholtz and Tscherning. Coleman proposes that the ciliary muscle contracts, releasing the tension exerted by the zonules and pulling the choroid forward. The choroid pushes the vitreous into the lens, supporting it; an idea first proposed by Cramer (1851). Intraocular pressure is not increased in the vitreous chamber, due to the opening of aqueous outflow by contraction of the longitudinal fibres of the ciliary muscle. These hydraulic forces cause a forward movement of the anterior surface of the lens while the position of the posterior lens surface remains fairly stable. According to Coleman, changes in lens shape are not caused by capsular elasticity as

claimed by Fincham (1937). Schachar *et al.* (1995) have resurrected Tscherning's

theory by claiming that zonular tension increases with ciliary muscle contraction, due to

a measured increase in the equatorial diameter of the eye with pharmacological

treatment. Schachar *et al.* propose that the tension exerted by the anterior and posterior

zonules is decreased while the zonular fibres attaching at the equatorial surface of the

lens become taunt.

The literature shows that debate still exists over the mechanism of

accommodation. An examination of the ciliary muscle and its action on the structures

involved in accommodation is fundamental to an understanding of accommodation and

may support or rule out some of the above theories. Changes in the ciliary muscle with

contraction have been noted in the literature: such as changes in the proportion of

muscle fibre types (Lütjen, 1966). However, these results have been complicated due to

species differences between humans and monkeys (see Section II). Many of the studies

of human ciliary muscle have been examining the changes in the ciliary muscle with age

(Tamm *et al.*, 1992b) and the majority are not anatomical (Swegmark, 1969; Strenk and

Semmlow, 1995). While some of these studies shed some light on the action of the

ciliary muscle in accommodation, the results cannot be directly compared to the results

obatined from anatomical studies with monkeys. Knowledge of the mechanism of

accommodation is also necessary in understanding the age-related loss of

accommodation: presbyopia. These issues will be addressed in Section II.

Birds

Birds are reported to have the largest accommodative amplitude in vertebrates,

with most birds having a range of 20 dioptres (Meyer, 1977) [A 20-year old human has

an accommodative range of approxiamtely 10 dioptres (Duane, 1922).] One of the reasons for this difference is that some birds appear to have two methods of accommodation: corneal and lenticular. During lenticular accommodation, the ciliary muscle contracts, decreasing the diameter of the ciliary body and forcing the ciliary processes onto the lens. This sphincter-like action increases the refractive power of the lens. There is some evidence that the iris may play a role in lenticular accommodation in birds as well (chickens, pigeons--Glasser *et al.*, 1995a and b; diving ducks--Levy and Sivak, 1980; Sivak, 1982; Sivak and Vrablic,1982). The peripheral iris contains muscle fibres which may squeeze the lens when contracted.

Descriptions of corneal accommodation suggest that when the ciliary muscle contracts, the peripheral edges of the cornea flatten. This peripheral flattening causes a central steepening of the cornea which increases corneal refractive power (Glasser *et al.*, 1994). Due to the large difference in refractive index between air and the cornea, very small changes in corneal shape can produce large changes in dioptric power. However, not all studies have been able to demonstrate changes in corneal curvature with accommodation (Levy and Sivak, 1980; Suburo and Marcantoni, 1983; Sivak *et al.*, 1986). The debate over the existence of corneal accommodation has continued for over a century and corneal accommodation has only recently received more widespread acceptance (see Section I, Introduction). The examination of the insertions and origins of the ciliary muscle fibres groups in the avian ciliary muscle will show whether the ciliary muscle is anatomically positioned to cause corneal accommodation, at least in some species.

It has been suggested that the bird eye is capable of retinal movement in the area of the fovea and that this assists in fine focusing an image on the retina (see Walls, 1942; Meyer, 1977; Munk, 1995). However, this possibility requires further investigations.

Thesis Objectives

This thesis addresses the functional anatomy of the ciliary muscle. The divisions of the ciliary muscle are examined and the role of these fibre groups in creating changes in the ciliary muscle, and consequently lens shape, are discussed. These points, while not addressed adequately in the literature, are fundamental to the understanding of accommodative function. The first half of this thesis (Section I) examines the ciliary muscle of birds. Here the ciliary muscle is striated and makes direct contact with the lens. This makes the avian ciliary muscle an ideal animal model to study the functional anatomy of the ciliary muscle. The second half of the thesis (Section II) examines the human ciliary muscle to determine how this smooth muscle is able to indirectly control accommodation. In both groups, the mechanism by which the ciliary muscle is able to produce lenticular changes is investigated by comparing the ciliary muscle in both the relaxed and contracted states. In the avian case, nicotine sulfate was used to induce contraction of the striated muscle fibres; whereas in the human eyes, atropine was used to relax the smooth muscle and pilocarpine to contract it. Both groups are also analyzed for asymmetries of the muscle around the circumference of the eye. This data

is used to evaluate whether the ciliary muscle contracts uniformly around the eye during accommodation. Four avian species with differing accommodative requirements (chickens, pigeons, kestrels, and hooded mergansers) were chosen in order to systematically assess the functional anatomy of the ciliary muscle in birds and to help clear up the confusion that exists concerning species differences. Among the human eyes, an analysis of the changes in ciliary muscle shape and of the relative proportions of muscle fibre groups with contraction is made. This study will further the understanding of the role of the human ciliary muscle in accommodation and address the differences in the mechanism of accommodation between humans and primates. These measurements are also analyzed across age to see how these changes may contribute to presbyopia (the age-related loss of the ability to accommodate). In summary, the main objective of this thesis is to describe the functional anatomy of the ciliary muscle in birds and humans and correlate the actions of the muscle in two groups in which accommodation involves change in the shape of the lens. In addressing this objective, species differences will be examined in both Sections. The possible role of the human ciliary muscle in presbyopia will also be examined.

Section I

The Functional Anatomy of the Ciliary Muscle in Four Avian Species

Introduction

Accommodation is the process by which the eye focuses on objects located at different distances. In vertebrates this process can occur by directly or indirectly applying force to change the curvature of the lens, by moving the lens, or by changing the curvature of the cornea. The mechanism of accommodation has been difficult to define due to the associated structures located within the eye; not visible to an observer. The ciliary muscle has been shown to be responsible for accommodation in terrestrial vertebrates, although in some avian species the iris muscles have also been shown to contribute to lenticular accommodation. The ciliary muscle is positioned between the peripheral cornea and the anterior choroid, forming a ring around the lens. Since it is difficult to observe the contractions of the ciliary muscle *in vivo*, the functional anatomy of the ciliary muscle is difficult to interpret.

The ciliary body of the eye is composed of the ciliary muscle, ciliary processes, and the pigmented and unpigmented epithelium associated with these structures (Hogan, 1971). The ciliary body can be divided into 1) the pars plana, from the border of the ora serrata to the ciliary folds and 2) the pars plicata, the area from the ciliary folds to the root of the iris. In avian species the ciliary muscle is not continuous with the epithelial layers of the ciliary body and ciliary processes as in humans (Tripathi, 1974). The avian ciliary body is divided by a ciliary cleft, separating it into an outer leaf composed of ciliary muscle, trabecular meshwork, and aqueous sinus and an inner leaf formed by the fibrous baseplate of the ciliary body (Tripathi, 1974) (fig. 1a). The ciliary epithelial layers and ciliary processes are positioned on the internal surface of this baseplate. The

Figure 1: A representative avian ciliary muscle at low-magnification to show ciliary

muscle orientation. a) Light micrograph of the chicken ciliary muscle (nasally) in a

contracted state showing the cornea (c), iris (i), ciliary processes (cp), baseplate of the

ciliary body (b), ciliary muscle (cm) and scleral ossicles (o). b) A tracing of the ciliary

muscle to distinguish the different main muscle fibre groups: anterior (A), posterior (P),

and internal (I). The lens (L) has also been drawn in to show its orientation in respect to

the ciliary processes and ciliary muscle.

0.25 mm
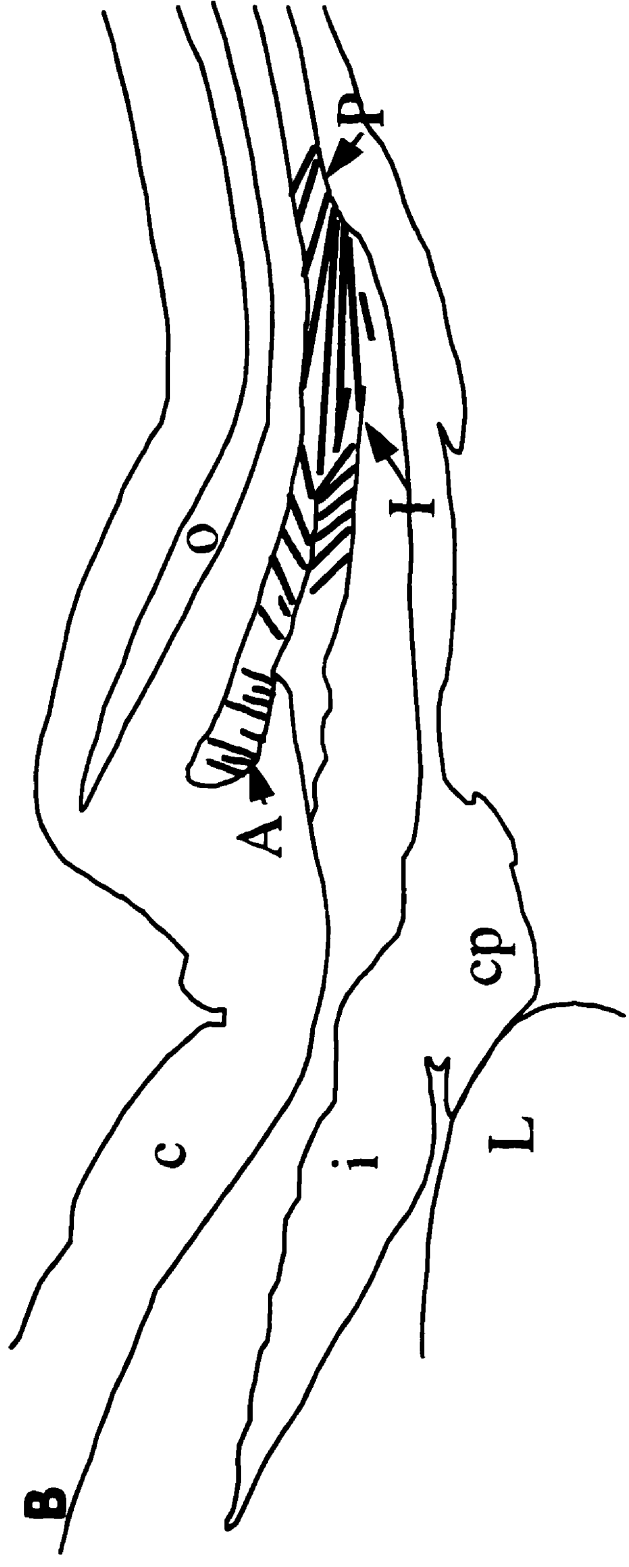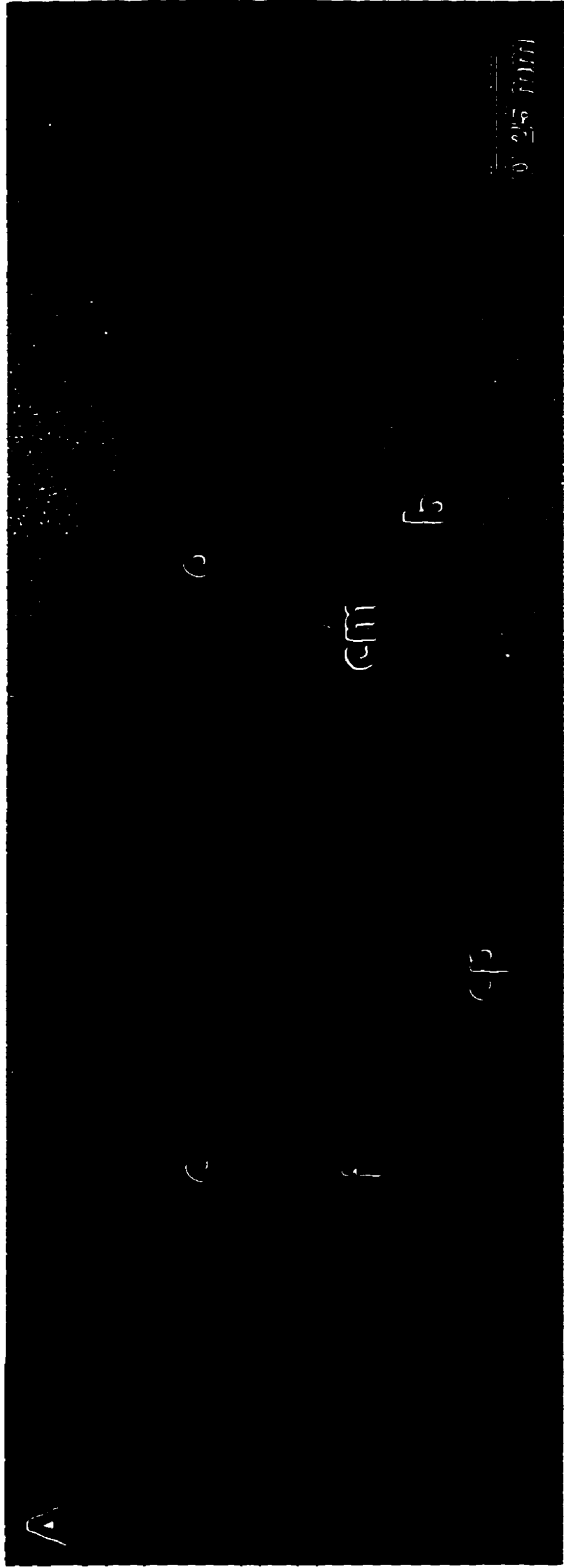
avian ciliary muscle is a striated muscle, although it is still innervated by the autonomic nervous system (Walls, 1942). The lens is surrounded by a ring of columnar epithelial cells called the annular pad (Willekens and Vrensen, 1985). The ciliary processes make direct contact with the annular pad. The avian eye contains a ring of overlapping bones called the scleral ossicles. This ring is contained within the sclera, positioned over the ciliary region of the eye and is responsible for the formation of the corneo-scleral sulcus (Walls, 1942).

Birds can accommodate by changing the shape of the lens and cornea. Lenticular accommodation can be described as the process by which the ciliary muscles contract, forcing the ciliary processes to squeeze the lens, decreasing its radius of curvature and thus increasing its power (Meyer, 1977; Levy and Sivak, 1980; West *et al.*, 1991). However, the role of the ciliary muscle in avian lenticular accommodation has been controversial due to the presence of muscle fibres in the iris periphery that have been shown to play some role in lenticular accommodation (Cramer, 1853; Müller, 1857; Hess, 1912; Glasser *et al.*, 1995). (See Glasser and Howland (1995a) for a historical review of avian accommodation.)

The cornea produces the major refractive surface in the eye (Helmholtz, 1909) and so small changes in corneal curvature can produce large refractive changes. The difficulty in detecting these corneal changes probably accounts for much of the controversy surrounding corneal accommodation in birds. From Crampton's first suggestion of the cornea playing a role in accommodation in birds (Crampton, 1813), the literature is filled with examples of positive and negative evidence of this process (Positive: Beer, 1893; Gundlach *et al.*, 1945; Goodge, 1960; Schaeffel and Howland,

1987; Troilo and Wallman, 1987; Glasser *et al.*, 1994, 1995. Negative: Cramer, 1853; Levy and Sivak, 1980; Suburo and Marcantoni, 1983; Sivak *et al*, 1986). Changes in corneal curvature have been reported for the chicken by stimulating the eye chemically (Troilo and Wallman, 1987) and electrically (Troilo and Wallman, 1987; Glasser *et al*, 1994). The pigeon eye is also reported to have chemically induced corneal changes (Gundlach *et al.*, 1945). Schaeffel and Howland (1987) observed corneal changes in both the pigeon and chicken during natural accommodation using photokeratometry. Various raptors have been shown to be capable of corneal change by placing pins in the cornea and observing the movement of the pins with accommodation (Beer, 1893). Aquatic birds that forage underwater would not benefit from corneal accommodation due to the loss of corneal power under water (Sivak and Vrablic, 1982). Therefore diving birds need a superior level of lenticular accommodation to produce the lens changes required to compensate for the loss of corneal refractive power while diving.

The anatomy of the ciliary muscle varies among avian species (Müller, 1857; Lord, 1956). However, three divisions of the ciliary muscle are often described: Crampton's muscle, Müller's muscle and Brücke's muscle (Gundlach *et al.*, 1945; Meyer, 1977; Schaeffel and Howland, 1987). Crampton (1813) first described the ciliary muscle in eagles and ostriches as a muscle arising from the internal surface of the sclera under the sclera ossicles and inserting into the internal surface of the cornea. Brücke (1846), examined owls and emus and verified the existence of Crampton's muscle and described a second group of fibres posterior to Crampton's muscle that course backward in the eye, originating under the scleral ossicles and inserting into the choroid. Brücke (1846) refers to this muscle as the *tensor choroidea*, theorizing that it

tenses the choroid in order to pull it anteriorly towards the lens. In addition to Brücke's

tensor choroidea, Müller (1857) describes a second posterior fibre group in the falcon

eye that runs from the choroid to insert indirectly into the inner lamellae of the cornea.

Since the initial description of these muscle groups in the 1800's, much

confusion has arisen as to the exact divisions of the ciliary muscle, in part because of

differences among species. Some authors have generalized in suggesting that the ciliary

muscle in all avian species consists of two divisions: Crampton's and Brücke's muscle

(Duke-Elder, 1958; Sivak, 1980). Other authors have described the avian ciliary muscle

as having three parts: Crampton's, Brücke's and Müller's muscles (Gundlach *et al.*,

1945). More confusion arises as to the exact location of Müller's muscle. It has been

referred to both as the posterior portion of Crampton's muscle (Walls, 1942) and as the

anterior portion of Brücke's muscle (Meyer, 1977; Levy, 1979). It is unclear whether

Müller's muscle is a separate muscle group or simply a subdivision of Crampton's or

Brücke's muscles.

As noted, the divisions of the ciliary muscle are not uniform among the avian

species. Lord (1956) reported on six species of birds with varying numbers of muscle

divisions. These included a single group of ciliary muscle fibres in the meadowlark to

four groupings in the sparrow-hawk and red-tailed hawk consisting of Crampton's,

Müller's, Brücke's, and a newly described temporal muscle. The diving ducks are

reported to have few or no anterior fibres (Walls, 1942). Slonaker (1918) describes the

ciliary muscle of the English sparrow as one unit without divisions. The chicken ciliary

muscle has been described as having an anterior and posterior muscle group (Suburo

and Marcantoni, 1983; West *et al.*, 1991), or having two regional groups with five

distinct arrangements of muscle fibres (Murphy *et al.,* 1995).

In this study, the ciliary muscle of four avian species with varying

accommodative needs were examined: chickens (*Gallus gallus domesticus*), pigeons

(*Columbia livia*), kestrels (*Falco sparverius*), and hooded mergansers (*Margus*

cucullatus). The ciliary muscles of each species was studied in both the relaxed and

contracted state in an effort to describe the functional anatomy of the avian ciliary

muscle. A detailed analysis of the muscle fibre groups was carried out to reveal

similarities and differences between species. The muscle fibre groups are compared to

the historical groupings and renamed according to their location within the ciliary

muscle, thereby helping to diminish the existing confusion surrounding the divisions of

the avian ciliary muscle.

Methods

The eyes of sixteen chickens (*Gallus gallus domesticus*), seven white and silver king pigeons (*Columbia livia*), seven American kestrels (*Falco sparverius*) (also known as sparrow hawks), and two hooded mergansers (*Margus cucullatus*) were examined. All birds were young adults with fully developed accommodative systems.

The birds were sacrificed by asphyxiation with carbon dioxide or by decapitation. A small knot was sewn in the connective tissue covering the sclera just adjacent to the cornea to mark the superior orientation in all birds except the hooded mergansers. (The hooded merganser eyes were collected early in the study, before orientation of the eye was known to be critical.) The eyes were then carefully enucleated. One eye of each chicken, pigeon, and kestrel was equatorially bisected (frontal section through half of the eye) and the anterior segment placed in 20% nicotine sulfate (ICN Biomedicals Canada, LTD) for approximately five minutes. This concentration has previously been used to induce strong pupillary constriction and accommodation (West *et al.*, 1991). In the hooded merganser the nicotine sulfate solution was applied by dropper to the corneal surface of intact enucleated eyes for approximately five minutes. The contralateral eye of each bird was assumed to be in a relatively relaxed state and was used as the control eye. All eyes were immediately fixed in either Bouin's fluid or 10% buffered formalin.

After fixation for at least one week, each control eye was carefully dissected to remove the posterior sclera and retina of the eye, the vitreous humour, and the lens. In the nicotine treated eyes the vitreous humour and the lens were removed. The anterior

portions of all eyes were cut radially into eight equal pie-shaped wedges, containing the ciliary body, cornea, and the iris. These wedges will be referred to as superior (S), superior nasal (SN), nasal (N), inferior nasal (IN), inferior (I), inferior temporal (IT), temporal (T), and superior temporal (ST) (fig. 2). All wedges were dehydrated through a graded series of alcohols and embedded in paraffin. The orientation of each wedge was carefully monitored during embedding. Serial sections (6-7µm) were cut on a standard rotary microtome to obtain about 40 sections per wedge, or approximately 320 sections per eye. The sections were stained with Milligan's trichrome stain (Humason, 1962). Histological sections were examined and photographed using bright field microscopy on a Nikon (Labophot-2A) light microscope or a Wild Leitz Binocular microscope.

Three pairs of eyes from the chickens, pigeons, and kestrels, and two pairs from the hooded mergansers were studied quantitatively. Five sections from each wedge were examined, yielding a total of 40 sections per eye and a statistical analysis was done on all measurements. The following measurements were made using an ocular micrometer: total muscle length, anterior cross-sectional muscle width (measured at the middle of the anterior fibre groups) and posterior cross-sectional muscle width (measured at the middle of the internal fibre group) , muscle fibre length for each muscle grouping, number of muscle fibres in each muscle grouping as seen in cross-section, the length along the baseplate of the ciliary body where muscle fibres were attached (fibre area on baseplate), and ciliary body baseplate length from the iris to the start of the fibre attachments (baseplate length). The muscle fibre length and numbers were measured from fibres that appeared parallel to the plane of sectioning. Standard

Figure 2: Sketch of the wedge positioning after dissection of the anterior segment of the

avian eye.

Superior (S)

Superior nasal (SN)

Superior temporal (ST)

Nasal (N)

Temporal (T)

Inferior nasal (IN)

Inferior temporal (IT)

Inferior (I)
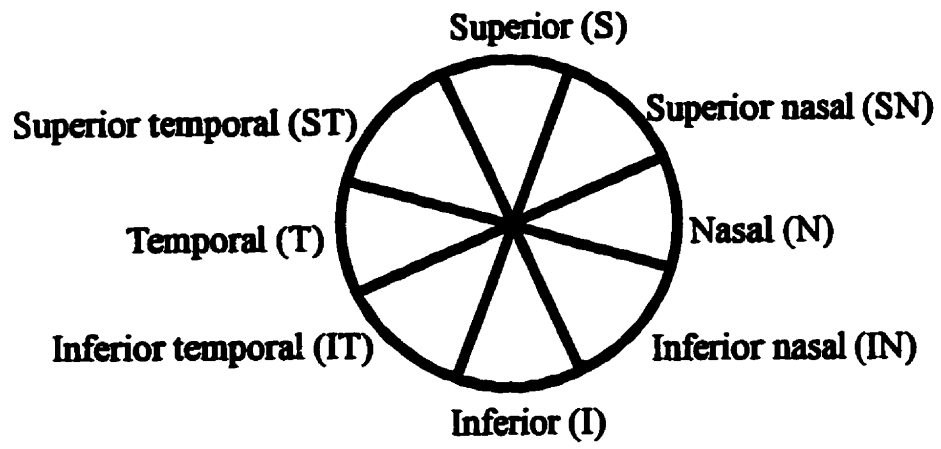
paired t-tests were used to compare the treated versus the control eyes for each measurement. A probability value equal to or less than 0.05 was considered significant.

Nasal-temporal asymmetry of the avian eye has been previously reported (Walls, 1942; Murphy *et al.*, 1995). The kestrel, pigeon, and chicken eyes were analysed for asymmetries of the ciliary muscle common to all three species, unique to each species and for asymmetric contractions of the ciliary muscle. The hooded merganser eyes were not used due to uncertainty of wedge orientation. To test for these asymmetries a three-way repeated measures ANOVA was performed on the interaction of wedge versus treated and untreated eye (eye) versus species for each measurement listed in the previous paragraph. The Greenhouse-Geisser epsilon was used to correct for any asphericity in the data ($\alpha < 0.05$) (Greenhouse and Geisser, 1959). All significant interactions were analysed using simple effects ($\alpha < 0.05$) (Howell, 1992). To examine the main effect, linear contrasts were performed between the nasal, nasal inferior, and/or nasal superior regions and the temporal region of the eye ($\alpha < 0.025$) (Howell, 1992).

Results

The ciliary muscle of all four avian species studied can be divided into three main groups based on insertions and origins (fig. 1a and b). The anterior fibre group originates at the sclera under the scleral ossicles and inserts into the inner lamellae of the cornea at the limbus. This group appears to have up to 3 subdivisions in the different species, based on variation in fibre orientation between the relaxed and contracted states. The posterior fibre group also originates on the sclera, posterior to the origin of the anterior fibres, and inserts *posteriorly* onto the baseplate of the ciliary body. The third group, the internal fibre group, attaches between the baseplate of the ciliary body and the collagen strands extending into the lamellae of the cornea. In the chicken, pigeon, and kestrel ciliary muscles, the internal fibre group is subdivided into an anterior and posterior portion based on fibre location.

Chicken Muscle Fibre Groups and Subdivisions

The chicken ciliary muscle appears to be unified, since there are no spaces between the muscle fibre groups (fig. 3). The chicken ciliary muscle is 1.69 ± 0.03 mm long in the relaxed state from the most anterior and posterior insertion points of all muscle fibres. The muscle measures 1.52 ± 0.02 mm in the contracted state ($\Delta=10\%$, paired t-test: $t=8.34$, $p<0.001$)(table I and II). In both the relaxed and contracted states the anterior muscle width is 0.09 ± 0.001 mm. The posterior muscle width is 0.13 ± 0.003 mm in the control eye and narrows in the contracted state to 0.11 ± 0.002 mm ($\Delta=20\%$; $t=12.21$, $p<0.0001$).

Figure 3: A light micrograph showing the ciliary muscle of the chicken eye in the nasal

region in both the relaxed (A) and contracted (B) state. The chicken ciliary muscle is

composed of an anterior muscle fibre group (AFG) that is subdivided into α, β, and γ.

The anterior fibres originate at the sclera (S) under the sclera ossicles (O) and insert into

the inner lamellae of the cornea (C). The posterior muscle fibre group (PFG) originates

on the sclera and inserts onto the baseplate of the ciliary body (B). The internal muscle

fibre group (IFG) extends from the baseplate of the ciliary body to the inner lamellae of

the cornea and is subdivided into an anterior internal (a) and a posterior internal group

(p).

PFG

0.2 mm

A

PFG

0.2 mm

B

Table I: Control (relaxed) versus treated (contracted) ciliary muscle measurements (mm) in four avian species.

Measurement	Chicken		Pigeon		Kestrel		Hooded Merganser	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Muscle Length	1.69±0.03	1.52±0.02*	1.96±0.03	1.64±0.03*	2.64±0.04	2.43±0.04*	2.33±0.03	2.13±0.03*
Muscle Width-Ant.	0.09±0.001	0.09±0.001	0.16±0.004	0.13±0.004*	0.08±0.002	0.07±0.001*	0.28±0.02	0.29±0.01
Muscle Width-Post.	0.11±0.002	0.13±0.003*	0.06±0.002	0.07±0.002*	0.06±0.002	0.08±0.003*	0.15±0.01	0.17±0.01*
Fibre Length-								
Anterior α	0.07±0.002	0.06±0.002*	0.22±0.01	0.09±0.004*	0.19±0.01	0.08±0.003*	0.22±0.02	0.17±0.01*
Anterior β	0.18±0.01	0.15±0.01*	0.39±0.02	0.20±0.01*	0.21±0.01	0.12±0.01*		
Anterior δ	0.41±0.01	0.36±0.01*			0.26±0.01	0.15±0.01*		
Posterior	0.31±0.01	0.28±0.01*	0.27±0.01	0.21±0.01*	0.17±0.01	0.16±0.01	0.49±0.04	0.49±0.04
Ant. Internal	0.14±0.01	0.12±0.01	0.14±0.01	0.10±0.01*	0.18±0.02	0.11±0.01*	0.96±0.08	0.90±0.04
Post. Internal	0.26±0.01	0.25±0.01	0.32±0.02	0.21±0.01*	0.26±0.01	0.14±0.01*		

*Signifies that the treated parameter is significantly different from the control as tested by a paired t-test at a 0.05 level of significance.

Table II: The difference in muscle dimensions between the control and the treated eye, shown as percent change (control-treated).

Parameter	Chicken	Pigeon	Kestrel	Hooded Merganser
Muscle fibre length				
anterior α	16	58	56	21
anterior β	16	49	44	
anterior δ	10		40	
posterior	9	23	7	0
internal anterior	12	29	36	8
internal posterior	7	34	47	
Total Muscle Length	10	14	8	9
Muscle Width				
anterior	0	19	15	2
posterior	-20	-18	-25	-13

The anterior fibre group contains the largest percentage of fibres (66%) of the

chicken ciliary muscle (table III). Based on fibre orientation, the anterior fibre group in

the chicken can be divided into three subgroups which will be called α, β, and γ (fig. 3).

The fibres of the subgroups rotate from lying parallel to the scleral surface in the relaxed

state to approaching a more perpendicular position in the contracted state. The most

anterior subgroup, α, changes the most followed by the β subgroup, with the γ subgroup

changing the least. The anterior α fibre group consists of 8-9 fibres (table III) that

originate at the sclera and insert individually into the inner lamellae of the cornea.

These are the shortest fibres; 0.07±0.002 mm in the relaxed state and 0.06±0.002 mm in

the contracted state (table I). When these fibres contract, by approximately 16%

($t=4.68$, $p<0.0001$)(table II), the fibre orientation changes from about 45° to a more

perpendicular position with respect to the scleral surface. The anterior β fibre group

contains 19-20 fibres in cross-section (table III). These fibres originate on the sclera just

posterior to the anterior α fibres and insert individually into a collagen sheath that

extends from the innermost lamellae of the cornea along the internal surface of the

ciliary muscle. These fibres are 0.18±0.006 mm long in the relaxed state and form an

angle of approximately 45° with respect to the scleral surface. Upon contraction, the

fibres shorten by 16% (0.15±0.006 mm; $t=3.50$, $p<0.001$) and are angled at

approximately 25° in relation to the sclera. The anterior γ fibres lie closer to the sclera

than the previously described anterior groups and are oriented at an angle of about 10°

relative to the sclera in the relaxed state. This angle increases to about 20° in the

contracted state. These fibres originate on the sclera posterior to the anterior β fibres

Table III: The number of muscle fibres in the ciliary muscle of four avian species.

Muscle Fibre Groups	Chicken	Pigeon	Kestrel	Hooded Merganser
Anterior (total)	35.53 (66%)	27.47 (55%)	24.74 (62%)	7.03 ±0.24 (28%)
α	8.77 ±0.19	11.35 ±0.27	8.64 ±0.19	
β	19.59 ±0.42	16.12 ±0.33	8.21 ±0.22	
δ	7.18 ±0.22		7.88 ±0.32	
Posterior	6.75 ±0.15 (13%)	10.75 ±0.23 (22%)	4.85 ±0.14 (12%)	11.04 ±0.52 (29%)
Internal (total)	11.45 (21%)	11.33 (23%)	10.53 (26%)	20.61 ±0.63 (53%)
Anterior	4.71 ±0.23	4.29 ±0.16	3.14 ±0.21	
Posterior	6.74 ±0.28	7.03 ±0.25	7.39 ±0.29	

and insert into the collagen sheath extending from the inner lamellae of the cornea. The 7-8 fibres of this subgroup (table III) are the longest in the anterior fibre group and only shorten by approximately 10% (0.41±0.01 v 0.36±0.01 mm; t=2.71, p<0.01) during contraction.

The posterior fibre group originates on the sclera just posterior to the anterior γ fibres and inserts *posteriorly* onto the baseplate of the ciliary body (fig. 3). This group consists of approximately 6-7 fibres (table III) that lie parallel to the sclera and form no discernible subgroups. The posterior fibre group is the smallest with only 13% of the total number of muscle fibres in the chicken ciliary muscle. The fibres are 7.06±0.24 mm in length when relaxed and shorten by roughly 9% to 6.43±0.17 mm in the contracted state (t=3.25, p<0.002)(table I and II).

The internal fibre group extends from the collagen sheath that is continuous with the inner lamellae of the cornea to the baseplate of the ciliary body just anterior to the insertion point of the posterior fibre group (fig. 3). In the chicken the fibre area along the baseplate is 0.26±0.01 mm (the total length along the baseplate where the posterior and internal muscle fibres attach). The internal fibre group contains 21% of the total number of ciliary muscle fibres (table III). The internal fibre group can be divided into two subgroups based on fibre location: the anterior internal fibre group and the posterior internal fibre group, which are often joined by a collagen sheath. The 6-7 fibres of the posterior internal muscle fibre group (table III) are sometimes separated by the short ciliary nerve. The fibres are 0.26±0.01 mm long when relaxed and shorten insignificantly to 0.25±0.01 mm long when contracted (Δ= 7%; t=1.30)(table I and II). The anterior internal group contains 4-5 fibres (table III). This small, spindle-shaped,

subgroup extends between the internal surface of the cornea and the posterior internal

muscle fibre group by a collagen sheath. These fibres shorten insignificantly by about

12% when treated (0.14±0.01 v 0.12±0.01 mm; t=1.13).

Pigeon Muscle Fibre Groups and Subdivisions

The pigeon ciliary muscle has a unified appearance, like the chicken ciliary

muscle (fig. 4). The ciliary muscle is 1.96±0.03 mm in length when relaxed, with an

anterior width of 0.16±0.004 mm and a posterior width of 0.06±0.002 mm (table I).

Upon contraction, through the effect of nicotine sulfate, the ciliary muscle shortens to

1.64±0.03 mm (Δ= 14%; t=9.88, p<0.001)(table II). The anterior muscle width thins by

19% to 0.13±0.004 mm (t=6.06, p<0.0001) while the posterior width thickens by 18%

to 0.073±0.002 mm (t=4.57, p<0.0001) with treatment.

Like the chicken anterior fibre group, the pigeon anterior fibre group is the

largest in the ciliary muscle with 55% of the fibres (table III). The pigeon anterior fibre

group contains two subgroups: anterior α and β based on fibre orientation between the

relaxed and contracted states (fig. 4). The anterior α group, consisting of 11-12 fibres

(table III), originates at the sclera and inserts onto the lamellae of the cornea. The

muscle fibres are 0.22±0.01 mm long when relaxed and shorten by 58% to 0.09±0.004

mm (t=14.8, p<0.0001)(table I and II). In the relaxed state the muscle fibres are

orientated roughly 45° to the sclera, an angle which increases to about 65° in the

contracted state. The anterior β group also originates on the sclera, but the 16-17

fibres (table III) insert into a collagen sheath that is continuous with the inner lamellae

Figure 4: The pigeon ciliary muscle from the nasal region of the eye as seen in the

relaxed (A) and contracted (B) states. This muscle is divided into an anterior muscle

fibre group (AFG) that originates on the sclera (S) under the sclera ossicles (O) and

inserts into the inner lamellae of the cornea (C); a posterior muscle fibre group (PFG)

that originates at the sclera and insert onto the baseplate of the ciliary body (B); and an

internal muscle fibre group (IFG) that extends from the inner lamellae of the cornea to

the baseplate of the ciliary body. The anterior muscle fibre group is subdivided into an

anterior α and an anterior β group based on fibre orientation between the relaxed and

contracted states. The internal muscle fibre group is subdivided into an anterior internal

(a) and posterior internal group (p).

PFG

0.20 mm

A

IFG

P

AFG

PFG

0.20 mm

B

a.IFG

P

B

AFG

of the cornea. In the relaxed state, the anterior β fibres are 0.39±0.02 mm long and form a 30° angle with the sclera. In the contracted state, the fibres shorten to 0.20±0.01 mm (Δ= 49%; t=11.83, p<0.0001) and change their orientation to form an angle of about 45° to the scleral surface.

The posterior fibre group contains 10-11 fibres in cross-section, constituting 22% of the total number of pigeon ciliary muscle fibres (table III). These fibres originate at the sclera posterior to the anterior fibre group and insert *posteriorly* into the baseplate of the ciliary body (fig. 4). These fibres shorten by approximately 23% in the treated eye (0.21±0.01 v 0.27±0.01 mm; t=4.87, p<0.0001)(table I and II).

The pigeon internal fibre group contains 23% of the ciliary muscle fibres, similar in size to the posterior fibre group (table III). The internal fibre group has two attachment points via collagen strands: the baseplate of the ciliary body, just anterior to the posterior fibre group insertion, and a collagen sheath that is continuous with the inner lamellae of the cornea (fig. 4). The total fibre area along the pigeon baseplate is 0.39±0.01 mm. The internal fibre group can be further divided into subgroups based on fibre location: anterior and posterior internal fibre groups that are joined by collagen. The posterior internal fibre group is larger, consisting of 7-8 fibres that measure 0.32±0.02 mm when relaxed and 0.21±0.01 mm when contracted (a difference of approximately 34%; t=5.91, p<0.0001)(table I, II, and III). The smaller and more spindle-shaped anterior internal fibre group contains 4-5 fibres that are 0.14±0.01 mm long in the relaxed state and shorten to 0.10±0.01 mm (Δ= 29%; t=3.00, p<0.005).

Kestrel Muscle Fibre Groups and Subdivisions

The kestrel ciliary muscle is more spread-out along the sclera than that of the other three species studied (fig. 5). The anterior and posterior groups are separated in the temporal region of the eye (see asymmetry), which makes the muscle fibre groups very distinct. The ciliary muscle of the kestrel is 2.64±0.04 mm long when relaxed and shortens to 2.43±0.04 mm in the contracted state (Δ= 8%; t=6.39, p<0.0001) (table I and II). In the kestrel, the ciliary muscle width thins anteriorly, from 0.08±0.002 mm to 0.07±0.001 mm (Δ= 15%; t=5.98, p<0.0001), and widens posteriorly, from 0.06±0.002 to 0.08±0.003 mm (Δ= 25%; t=6.45, p<0.0001), with treatment.

As in the chicken and pigeon, the kestrel anterior fibre group contains the largest percentage of muscle fibres (62%) in the kestrel ciliary muscle (table III). The anterior fibre group in kestrels consists of three subgroups with different fibre orientations between the relaxed and contracted states: α, β, and γ (fig. 5). As in the chicken, the fibres rotate to become more perpendicular to the scleral surface in the contracted state with the more anterior subgroups showing the greater change. The anterior α fibre group consists of 8-9 fibres (table III) that lie approximately 45° to the sclera when relaxed and rotate to an angle of approximately 65° to the sclera when contracted. These fibres originate on the sclera and insert anteriorly into the inner lamellae of the cornea. The fibres measure 0.19±0.01 mm long in the control eyes and 0.08±0.003 mm when treated (Δ= 56%; t=9.95, p<0.0001)(table I and II). The anterior β fibre group also originates on the sclera but inserts into the inner lamella of the cornea via a collagen sheath. This group consists of approximately 8-9 fibres (table III). They

Figure 5: A cross-section of the kestrel ciliary muscle in the nasal region of the eye in the relaxed (A) and contracted (B) states. The ciliary muscle has a anterior muscle fibre group (AFG) that originates at the sclera (S) under the sclera ossicles (O) and inserts into the inner lamellae of the cornea (C). This anterior fibre group is subdivided into three subgroups based on fibre orientation; α, β, and γ. The posterior muscle fibre group (PFG) originates at the sclera and inserts into the baseplate of the ciliary body (B). The internal muscle fibre group (IFG) extends from the inner lamellae of the cornea to the baseplate of the ciliary body and is subdivided into an anterior internal (a) and posterior internal group (p).
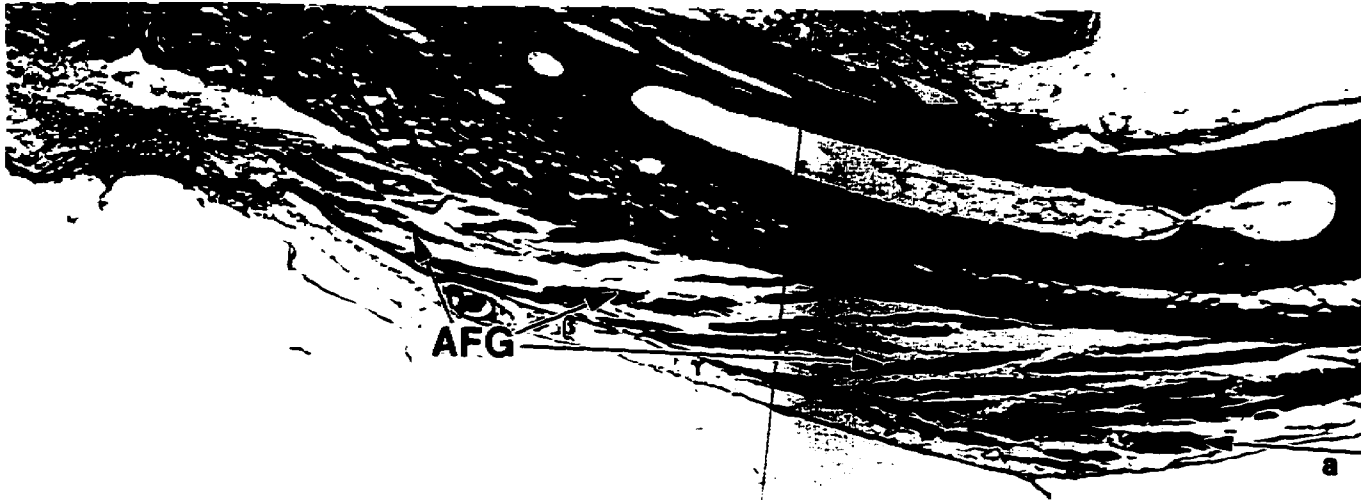
measure 0.21±0.01 mm in length when relaxed and 0.12±0.01 mm when contracted (Δ

= 44%; t=8.99, p<0.0001). The anterior β and γ fibres form a 30° angle with the sclera

when relaxed. The angle increases to 45° in the contracted state for anterior β fibres but

remains at 30° for the anterior γ fibres. The anterior γ fibre group originates just

posterior to the anterior β fibres on the sclera and inserts into the same collagen sheath

as the anterior β fibres that continue on to the cornea. This group contains 7-8 fibres

that shorten by 40% when treated (0.23±0.01 v 0.15±0.01 mm; t=8.13, p<0.0001)(table

III).

The posterior fibre group of the kestrel originates, as usual, at the sclera and

inserts posteriorly onto the baseplate of the ciliary body (fig. 5). The posterior fibre

group is the smallest of the kestrel ciliary muscle, containing 4-5 fibres or 12% of the

total number of fibres (table III). In the kestrel this group shortens insignificantly by

approximately 7% when treated from 0.17±0.01 mm to 0.16±0.01 mm (t=1.251) (table I

and II).

The internal fibre group contains an intermediate number of muscle fibres

between the anterior and posterior fibre groups (26%; table III). The internal fibre

group again can be described as having two subgroups: anterior and posterior. These

two groups are located in a collagen sheath extending from the inner lamellae of the

cornea to the baseplate of the ciliary body (fig. 5). The fibre area along the baseplate

due to the posterior and internal muscle fibres attaching is 0.19±0.01 mm. The anterior

internal group is small and spindle-shaped, containing 3-4 fibres that are 0.18±0.02 mm

long in the relaxed state (table III). Upon contraction the fibres shorten to 0.11±0.01

mm (Δ=36%; t=7.59, p<0.0001)(table I and II). The posterior internal group is more

prominent, consisting of 7-8 fibres. The fibres shorten by about 47% with treatment

(0.26±0.01 v 0.14±0.01 mm; t=3.28, p<0.002).

Hooded Merganser Muscle Fibre Groups and Subdivisions

The hooded merganser ciliary muscle has a bulky appearance due to the large

size of the posterior and internal fibre groups (fig. 6). The ciliary muscle is 2.33±0.03

mm long when relaxed and 2.13±0.03 mm long when treated (Δ=9%; t=4.90, p<0.0001)

(table I and II). The muscle becomes thicker in width both anteriorly and posteriorly

when contracted. Anteriorly, the ciliary muscle width is 0.28±0.02 mm in the relaxed

state and widens insignificantly to 0.29±0.01 mm in the contracted state (Δ=2%,

t=0.33). Posteriorly, the muscle width is 0.15±0.01mm in the relaxed state and widens

to 0.17±0.01 mm in the treated eye (Δ=13%, t=2.85, p<0.01). The hooded merganser

ciliary muscle has a unified appearance and only contains the three main muscle fibre

groupings: anterior, posterior, and internal. No change in fibre orientation was seen

between the relaxed and contracted states to distinguish subgroups in the anterior fibre

group. The internal fibre group also contained no subdivisions. Subgroups may have

been revealed if the hooded merganser ciliary muscle had reacted more strongly to the

nicotine sulfate. The hooded merganser muscle fibres contracted the least of the species

studied.

The anterior fibre group contains 7-8 fibres (table III) that originate along the

scleral surface and insert onto the collagen of the inner lamellae of the cornea. These

Figure 6: The hooded merganser ciliary muscle as seen in the nasal region of the eye in both the relaxed (A) and contracted (B) states. This ciliary muscle has the three main fibre groups but no subdivisions. The anterior muscle fibre group (AFG) originates at the sclera (S) under the sclera ossicles (O) and inserts into the inner lamellae of the cornea (C). The posterior muscle fibre group (PFG) originates at the sclera and insert onto the baseplate of the ciliary body (B). The internal muscle fibre group (IFG) extends from the periphery of the cornea to the baseplate of the ciliary body.

A

B
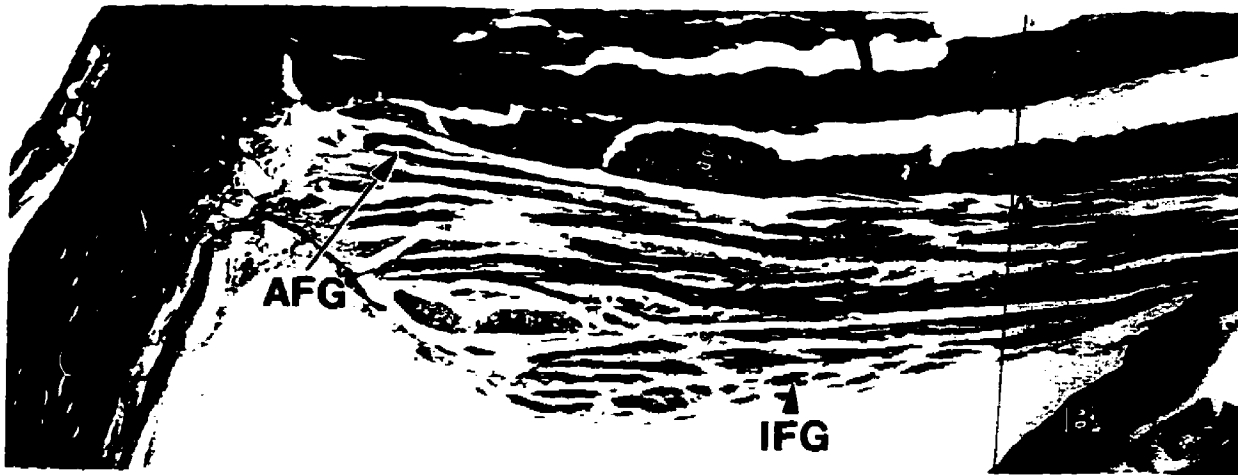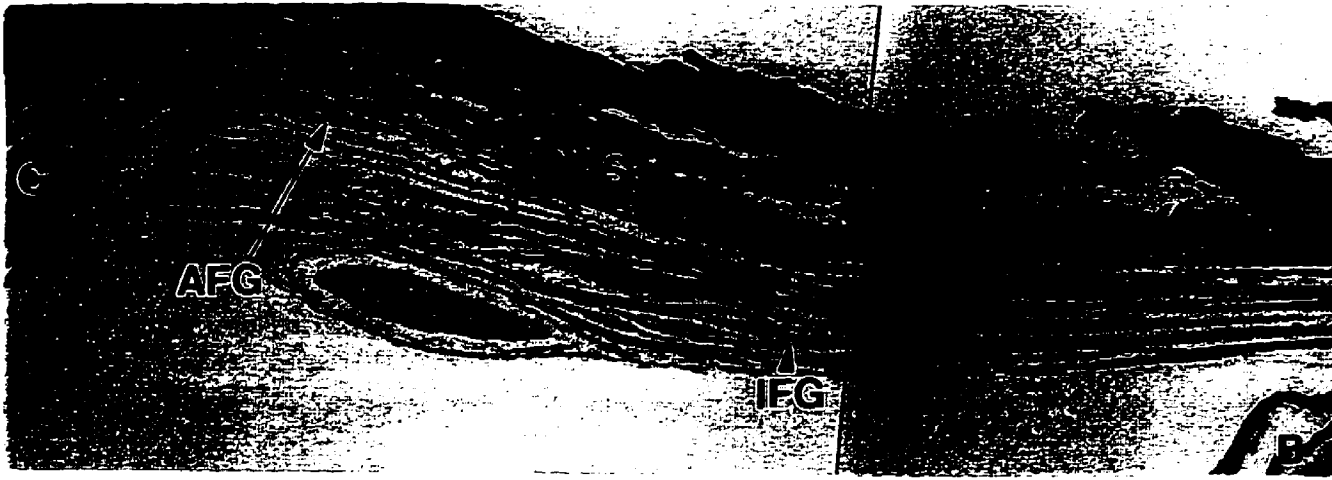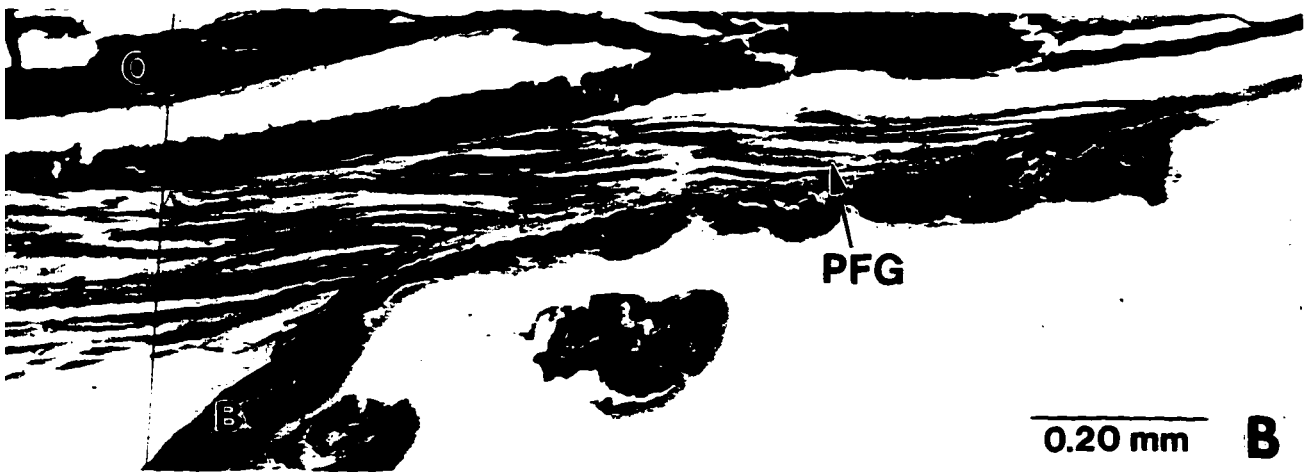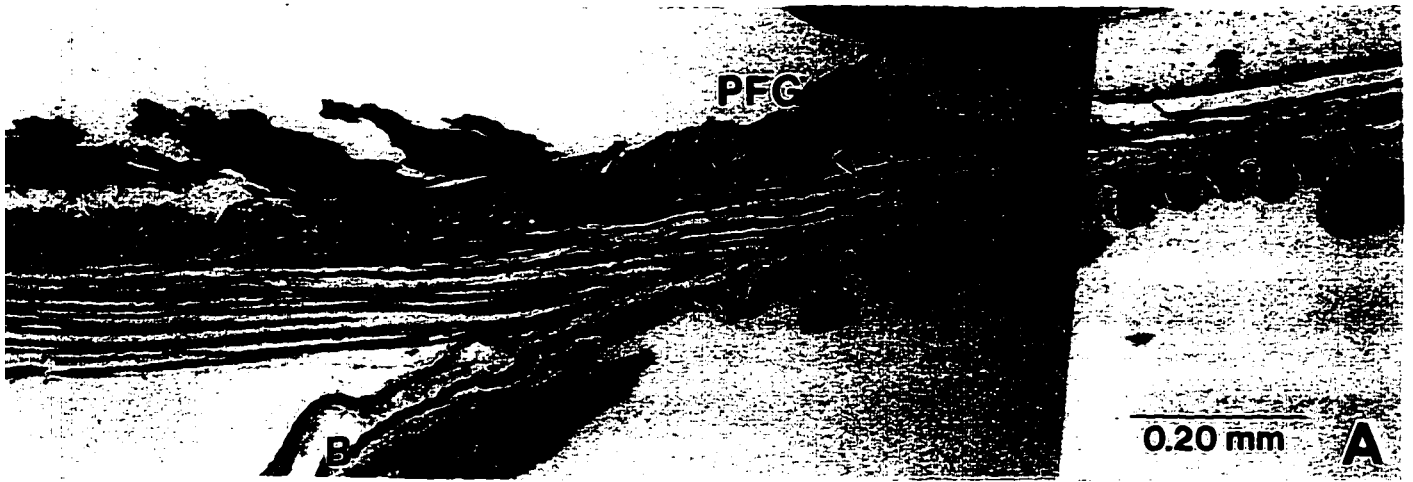
fibres shorten by about 21% with treatment (0.22±002 v 0.17±0.01 mm; t=56.4, p<0.02).

The posterior fibre group originates on the sclera just posterior to the anterior fibres and extends posteriorly to the baseplate of the ciliary body (fig. 6). This group contains 11-12 fibres in cross-section (table III). The anterior and posterior fibre groups are similar in size, the anterior fibre group containing 28% of the total number of fibres while the posterior fibre group contains 29% (table III). The fibres of this group did not show a significant change with treatment (0.49±0.04 v 0.49±0.04 mm; t=0.022) (table I and II). This is likely artifactual since the posterior portion of the eye was not removed and the nicotine sulfate may not have penetrated to the posterior fibre group.

The internal fibre group is the largest in the hooded merganser eye, containing 20-21 fibres or 53% of the total number of ciliary muscle fibres (table III). These fibres extend from the baseplate of the ciliary body, just anterior to the posterior fibre group insertion point, to collagen that is continuous with the inner lamellae of the cornea. This collagen attachment with the inner lamellae forms a "Y". The fibres attach to the inside "V" of the "Y" while the stem of the "Y" is continuous with the inner lamellae of the cornea (fig. 6). The fibres of the posterior and internal fibre groups attach along the baseplate of the ciliary body for 1.01±0.03 mm, the largest fibre area of the species studied. The internal fibres are 0.99±0.08 mm in length in the relaxed state and shorten insignificantly to 0.90±0.04 mm in the contracted state (Δ=8%; t=0.965) (table I and II).

Asymmetry

The ANOVA revealed that the three-way interaction of eye (treated versus control) by species by wedge is not significant for any measurement taken in the three

species tested: chicken, pigeon, and kestrel. The avian ciliary muscle appears to contract uniformly around the circumference of the eye for these species since the interaction of eye by wedge is not significant for total muscle length, muscle width, or individual fibre group lengths.

The total muscle length is asymmetrical around the circumference of the eye of the chicken, pigeon, and kestrel (table IV) (fig. 7a). The nasal portion of the muscle is significantly shorter than the temporal portion ($\bar{x}$=1.61 v $\bar{x}$=2.14 mm). The same asymmetrical pattern is also seen in the length of the ciliary body baseplate for these three species (table IV) (fig. 7b). The nasal portion of the baseplate ($\bar{x}$=1.49 mm) is shorter than the temporal portion ($\bar{x}$=2.55 mm). The length of the baseplate around the circumference of the eye is different between species (table IV) as shown by the interaction of eye by wedge. The ciliary body baseplate is relatively longer in the temporal region of the kestrel eye as compared to the temporal region of the chicken and pigeon eye ($\bar{x}$=3.31 mm v $\bar{x}$=2.11 and $\bar{x}$=2.23 mm, respectively).

The anterior ciliary muscle width is not significantly different around the circumference of the avian eyes tested (table IV). The width of the posterior ciliary muscle in the chicken shows no variation around the circumference of the eye (one-way repeated ANOVA: $F_{(7, 35)}$=1.675). The kestrel and pigeon ciliary muscles are significantly wider posteriorly in the nasal portion of the eye compared to the temporal portion. In the kestrel ciliary muscle the nasal region has a mean posterior width of 0.10 mm and the temporal region, a mean posterior width of 0.04 mm. The ciliary muscle in the chicken has a mean posterior width of 0.08 mm nasally and 0.06 mm temporally.

Figure 7: Plots of the length (mm) of the ciliary muscle (a) and ciliary body baseplate

(b) around the eye. Note the asymmetry in both measurements, with the nasal side the

shortest. Regions of the eye represented by S: superior, SN: superior nasal, N: nasal,

IN: inferior nasal, I: inferior, IT inferior temporal, T: temporal, and ST: superior

temporal.

a
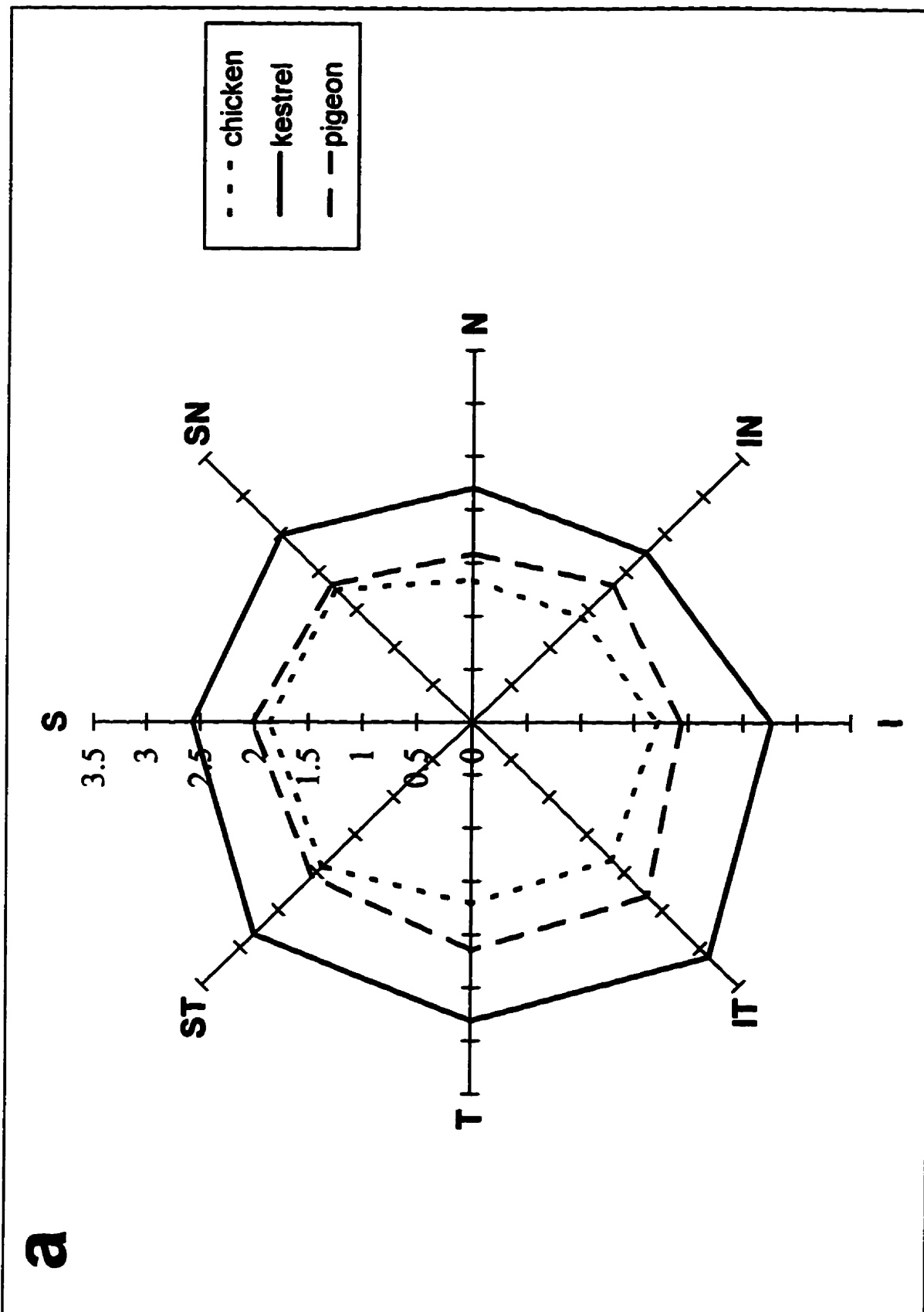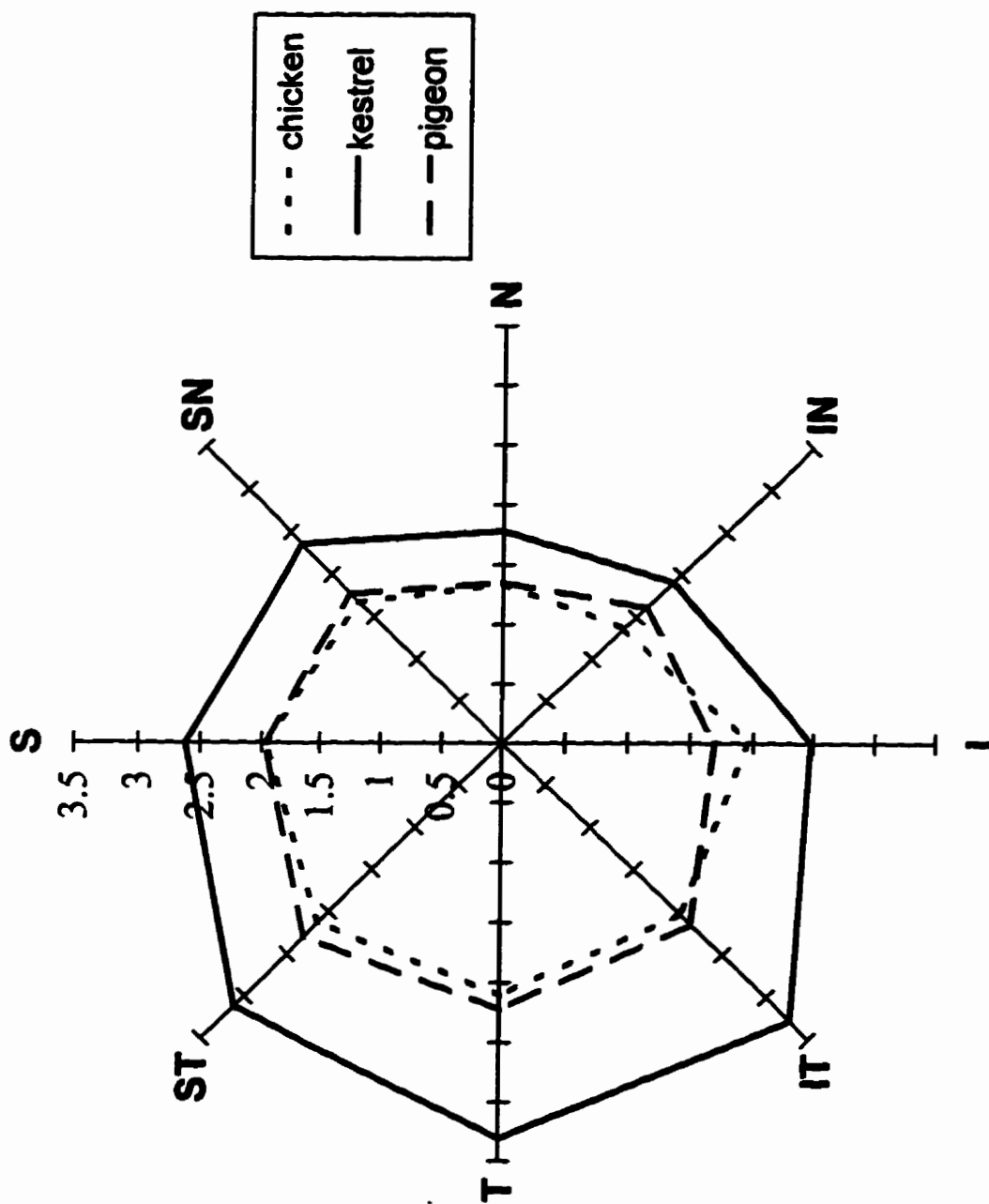
b

Table IV: Results of a three-way ANOVA performed on the interaction of control versus

Parameter		Source	Degrees of Freedom	F value (*p<0.05)
Muscle Length		Wedge	7, 42	24.84*
		Wedge X Species	14, 42	2.02
Baseplate Length		Wedge	7, 42	53.47*
		Wedge X Species	14, 42	4.80*
Fibre Length	α	Wedge	7, 42	1.50
		Wedge X Species	14, 42	1.11
	β	Wedge	7, 42	6.58*
		Wedge X Species	14, 42	1.62
	δ	Wedge	7, 28	1.09
		Wedge X Species	7, 28	4.58*
	posterior	Wedge	7, 42	1.40
		Wedge X Species	14, 42	0.77
	internal posterior	Wedge	7, 42	1.88
		Wedge X Species	14, 42	1.95
Fibre Numbers	α	Wedge	7, 42	3.74*
		Wedge X Species	14, 42	1.85
	β	Wedge	7, 42	4.29*
		Wedge X Species	14, 42	1.16
	δ	Wedge	7, 28	2.84
		Wedge X Species	7, 28	5.05*
	posterior	Wedge	7, 42	2.26
		Wedge X Species	14, 42	2.47
	internal posterior	Wedge	7, 42	4.78*
		Wedge X Species	14, 42	2.75
Width Anterior		Wedge	7, 42	3.03
		Wedge X Species	14, 42	2.61
Width Posterior		Wedge	7, 42	22.52*
		Wedge X Species	14, 42	11.42*

The fibre length for each muscle fibre group is symmetrical around the eye except for the anterior β and anterior γ muscle fibre groups (table IV). The fibres of the anterior β muscle fibre group are significantly longer in the IN region ($\bar{x}$ =0.22 mm) compared to the T region ($\bar{x}$ =0.18 mm) in all three species. The anterior γ fibre group is symmetrical around the circumference of the eye in the chicken (one-way repeated measures ANOVA, F(7,35)=3.244, p=0.063), while in the kestrel the nasal γ fibres ($\bar{x}$ =0.21 mm) are significantly longer than the temporal γ fibres ($\bar{x}$ =0.14 mm). (Note that the anterior γ fibre group only exists in the chicken and kestrel eyes.)

The number of muscle fibres seen cross-sectionally in the anterior α and β fibre groups is asymmetrical (table IV). In both fibre groups the SN region has significantly more fibres than the T region. In the anterior α fibre group, the SN region has a mean of 10.28 fibres and the T region a mean of 8.09 fibres. The anterior β fibre group contains a mean of 16.99 fibres in the SN region of the eye and a mean of 11.58 fibres in the T region. The number of anterior γ fibres around the circumference of the eye differ between the chicken and kestrel (table IV). The number of anterior γ fibres are symmetrical around the chicken eye (one-way repeated measures ANOVA, F(7, 35)=1.728). In the kestrel eye there are more anterior γ fibres in the nasal region compared to the temporal region ($\bar{x}$ =10.22 v $\bar{x}$ =4.75 fibres). The posterior fibres are consistent in number around the circumference of the eye for all three species. The internal posterior fibres are more numerous in the IN region ($\bar{x}$ =9.06) compared to the T region ($\bar{x}$ =5.11) of the eye in all species tested (table IV).

Upon visual inspection it is noted that the positioning of the ciliary muscle fibre groups is asymmetrical in the kestrel eye. The anterior fibre groups are separated from the posterior and internal fibre groups in the temporal and superior regions of the eye (fig. 8). In the nasal region the muscle fibre groups appear more unified (as in the other species studied) and the aqueous sinus is not present. The ciliary muscle also changes position with respect to the scleral ossicles. In the nasal region the muscle's anterior insertion point is approximately equal to the anterior point of the ossicles. The insertion point moves more anterior to the ossicles in the rest of the eye, reaching its most anterior point in the ST and S regions.

All of the fibre groups, except the anterior internal fibre group, are found in every region of the avian eye. In the chicken the anterior internal fibre group is found in the S and SN regions and is present sporadically in the I and IT regions. The kestrel anterior internal fibre group is seen in the N and I regions of the eye, being absent in the T, ST and S regions. The anterior internal fibre group of the pigeon can be seen in all regions of the pigeon eye.

Figure 8: A schematic of the asymmetry of the ciliary muscle fibre groups in the

kestrel eye. The diagram of the left (OS) and right (OD) eye to the right of each figure

shows the region of the eye from which the schematic is based. Note that the muscle

fibre groups become more uniform and the venous sinus disappears in the nasal region

of the eye.

Scleral ossicle

Scleral border

OD OS

Muscle fibre groups

Aqueous sinus

Baseplate of ciliary body
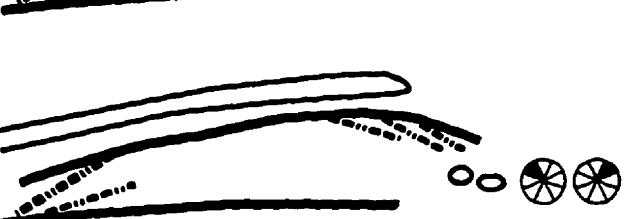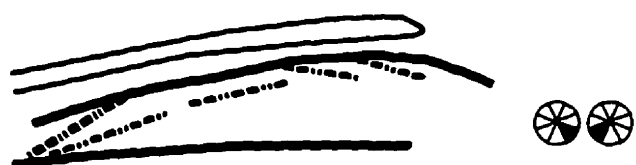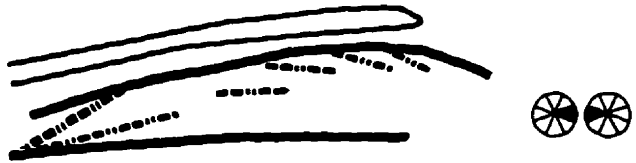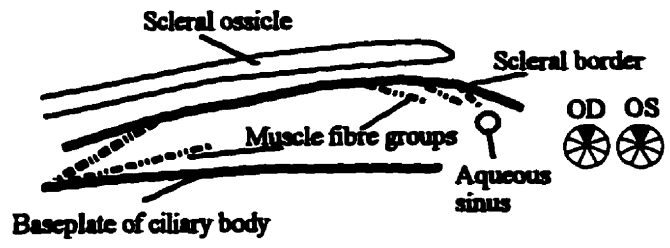
Discussion

This anatomical analysis of the avian ciliary muscle reveals that while there are similarities in the ciliary musculature of the four species, important differences do exist. These differences suggest that either corneal or lenticular accommodation is emphasized depending on the accommodative needs of these species.

Species Accommodative Needs

The species studied have varying accommodative needs. An accommodative range of 20 dioptres (D) is considered typical for the avian species that have been examined, with the greatest range found in aquatic birds (Meyer, 1977). Chickens and pigeons use between 9 to 25 D of accommodation (Gundlach et al., 1945; Schaeffel and Howland, 1987; and Troilo and Wallman, 1987; Glasser, et al., 1994) to peck at grain on the ground. The hooded merganser is a diving duck which pursues its prey underwater. Upon entering the water, the cornea of the eye loses its refractive power due to the anterior and posterior surfaces of the cornea being nearly parallel and the similar refractive indices between water and aqueous humour (Sivak, 1980). The lens of diving ducks bulges through the iris creating a lenticonus during accommodation (approximately 40-80 D) (cormorant, Hess, 1912; dipper, Goodge, 1960; and hooded merganser, Levy, 1979; Sivak et al., 1985), thereby compensating for the loss of corneal power. The kestrel may at first appear to require the least amount of accommodative power since it searches for prey from the sky (Terres, 1980). However, when stooping down to capture prey, this bird may need fine control of its accommodative mechanisms

for depth perception to keep the object of interest focused on the retina so as to not miss the target or dive into the ground (Lord, 1956). The kestrel is a semi-altricial species (Bird et al., 1984); since its young are helpless, the kestrel also needs to use accommodation to fixate the chick's mouth while feeding.

Role of the Ciliary Muscle in Accommodation

Based on the anatomical analysis presented here, the avian ciliary muscle can be divided into distinct groups and its role in corneal and lenticular accommodation deduced. Each avian species studied has three main muscle fibre groups: anterior, posterior, and internal (fig. 1 a and b). The anterior fibre group has historically been called Crampton's muscle (Crampton, 1813). The anterior fibres directly or indirectly insert into the inner lamellae of the cornea. This muscle group has been shown to draw the periphery of the cornea posteriorly about the fulcrum provided by the apex of the scleral ossicles (Glasser et al., 1994). This flattens the peripheral cornea and steepens the central cornea, increasing the refractive power. The posterior fibre group inserts onto the baseplate of the ciliary body, pulling it forward towards the cornea and thereby causing a reduction in lens radii of curvature (West et al., 1991). This group was first identified by Brücke (1846), who described the fibres inserting into the choroid. The internal fibre group corresponds to Müller's muscle and extends from the pars plana to the inner lamellae of the cornea (Müller, 1857). The internal fibres are consistent with an action that would pull the peripheral cornea posteriorly and the baseplate of the ciliary body anteriorly. Thus, the internal fibre group assists the action of the anterior

and posterior fibres simultaneously and contributes to both corneal and lenticular accommodation.

Recently, Murphy *et al.* (1995) have proposed a new nomenclature for the ciliary muscle of the chicken. They describe the chicken ciliary muscle as having two divisions with four anatomically distinct muscles. The anterior portion of the muscle is divided into an anterior and an intermediate ciliary muscle. These groups correspond to the anterior muscle fibre group and anterior internal muscle fibre group of the current study. Murphy *et al.* (1995) divided the posterior portion of the chicken ciliary muscle into two groups: a sinociliary muscle and a sclerociliary muscle. The sinociliary muscle corresponds to this study's posterior internal muscle fibre group while the sclerociliary muscle is equivalent to the posterior muscle fibre group. The current study divided the ciliary muscle into muscle groups based on the same origin and insertions for each grouping. The muscle fibre groups described here reveal the similarities in the four species studied.

The peripheral iris muscle has been reported to be the major force in squeezing the lens in chickens (Glasser *et al.*, 1995). The four species studied here have muscle fibres in the periphery of the iris. The largest number of peripheral iris muscle fibres appears to exist in the hooded merganser, followed by the chicken, the pigeon and then the kestrel with the least. In the hooded merganser the iris sphincter muscle may help to create a rigid ring against which the lens can be pushed to create a lenticonus (Levy and Sivak, 1980; Sivak and Vrablic, 1982).

The collagen of the ciliary body was clearly visible with the Milligan's trichrome stain, making it possible to gain a general impression of collagen content in the

baseplate of the ciliary body. The collagen strenghtens the baseplate, creating a firm, circumferential structure around the lens. When the ciliary muscle contracts, the basplate is pulled forward into the lens, squeezing it. The baseplate of the hooded merganser eye has the most abundant collagen relative to the other species studied. The pigeon baseplate was also fibrous having a thick layer of collagen. The eye of the kestrel appeared to have little collagen along the baseplate, giving it a thin appearance. The chicken baseplate had little to no collagen, but did not appear as compact as the kestrel ciliary body baseplate. This difference in abundance of collagen in the baseplate, in addition to the variation in peripheral iris muscles, suggests that different species have differences in accommodation.

Both the peripheral iris muscle and the posterior and internal muscle fibre groups of the ciliary muscle are probably involved in lenticular accommodation to varying degrees, depending on the species. Glasser *et al.* (1995) propose that the chicken accommodates lenticularly by contracting the peripheral iris muscle which applies a sphincter-like force to the ciliary processes that are positioned internal to the periphery of the iris. They also believe the baseplate is not rigid enough to exert force on the lens. They conclude that the contraction of the posterior and internal muscle fibres pulls the baseplate forward to release resting tension on the lens, and allow the peripheral iris muscle to squeeze the lens via the ciliary processes. The current study indicates that the baseplate of the ciliary body in the chicken is not rigid enough to exert any force on the lens, since the baseplate contained very little collagen. However, it seems likely that the baseplate may exert some force on the lens, particularly in the pigeon and hooded merganser.

The pigeon and chicken have similar accommodative needs, but their ciliary body morphology is different. While the chicken has a relatively large peripheral iris musculature and a thin baseplate, the pigeon peripheral iris muscle is small and the ciliary body baseplate is thick with collagen. It has been reported that the chicken ciliary processes attach anterior to the equator of the lens (Suburo and Marcantoni, 1983; West *et al*, 1991; Glasser and Howland, 1995b). The pigeon ciliary processes, however, insert at the equator of the lens and the iris peripheral muscle is not directly external to the processes but is situated more anteriorly (Glasser and Howland, 1995b). These differences suggest that contraction of the posterior and internal muscle fibre groups in the pigeon may pull the more rigid baseplate of the ciliary body forward. The ciliary processes would, therefore, be in a position to effectively change the shape of the pigeon lens.

The kestrel appears to have the least amount of lenticular accommodation of the species studied since few peripheral iris muscles are seen and the baseplate of the ciliary body is very thin. There is no anatomical evidence that the kestrel can achieve large changes in lens curvature. The hooded merganser, on the other hand, has the largest structures associated with lenticular accommodation of the species studied: an abundance of collagen in the baseplate and a large number of iris peripheral muscle fibres. The hooded merganser needs the greatest change in lens curvature to compensate for the loss of corneal power in water.

The width of the ciliary muscle increases posteriorly by 13 to 25% in all the species studied (table II). The change in width of the ciliary muscle during accommodation may alter the angle at which the baseplate of the ciliary body is pulled

into the lens (fig. 1a and b). In the kestrel and pigeon, the anterior width thins (19 and 15%, respectively) while the posterior width widens with treatment, possibly tilting the posterior portion of the baseplate slightly internal to its resting position. In the hooded merganser and chicken, the entire muscle widens. This suggests a smaller change in the angle of the baseplate occurs as it is pulled into the lens. This change in width, although a secondary effect of muscle contraction, suggests the complexity of the accommodative system.

Species Differences

The anatomy of the ciliary muscle in these species reflects their different accommodative needs. Anatomically, the ciliary muscle in the chicken and pigeon appears to be emphasized in the anterior region implicating a role in corneal accommodation (fig. 3 and 4)(table III). The largest percentage of fibres, as measured cross-sectionally, is found in the anterior fibre group of both species (66% in the chicken and 55% in the pigeon). The posterior fibres account for 13% of the total number of fibres in cross-section in the chicken and 22% in the pigeon. The internal fibre group may also have some influence on the cornea. In the chicken, the internal fibres constitute 21% of the total number of fibres, while in the pigeon internal fibres constitute 23%.

The relative proportions of ciliary muscle fibres in the kestrel are similar to that of the chicken and pigeon (fig. 5 and table III). The anterior fibres comprise 62%, the posterior fibres 12% and the internal fibres 26% of the total number of fibres seen in cross-section. Corneal accommodation was examined *in vivo* in two kestrels to see if

any changes in the curvature of the cornea could be detected. The spots of a Haag-Streit keratometer moved as the kestrel focused on near objects, indicating the presence of corneal accommodation. Beer (1893) observed corneal changes in raptors by placing pins in the surface of the cornea and observing their movement during accommodation.

It has been suggested that diving birds have no need for corneal accommodation, since the cornea loses its refractive power underwater (Levy and Sivak, 1980). The anatomy of the hooded merganser ciliary muscle seems to support this claim. The anterior fibre group is the smallest portion of the hooded merganser ciliary muscle, constituting 28% of the total number of fibres (table III). Thus, the hooded merganser ciliary muscle contributes less to corneal accommodation than the muscle of the other three species studied. Previous researchers have reported a reduced anterior fibre group in aquatic birds (Walls, 1942; Sivak, 1980). In the hooded merganser eye, the posterior fibres comprise 29% of the total fibres and the internal fibres 53%. Both of these groups contribute to lenticular accommodation. The fibre area along the baseplate of the ciliary body is also greatest in the hooded merganser. The fibres of the hooded merganser attach along the baseplate for a distance of 1.01 ± 0.03 mm, while the fibre area is 0.26 ± 0.01 mm in the chicken, 0.39 ± 0.01 mm in the pigeon, and 0.19 ± 0.01 mm in the kestrel. Therefore, it is likely that in the hooded merganser, the action of the ciliary muscle can force the thick baseplate of the ciliary body against the annular pad of the lens to form a lenticonus.

Asymmetry

Although nasal asymmetry has been noted in birds, it has not been well described (Walls, 1942; Lord, 1956). The chicken eye has been reported to have asymmetry in the anterior segment of the eye with the distance from the equator of the globe to the limbus being greatest temporally (Murphy *et al.*, 1995). This study shows that the ciliary muscle length and baseplate length in the chicken, kestrel and pigeon are shortest in the nasal region (fig 7). In the kestrel and pigeon eye, the posterior portion of the ciliary muscle is wider in the nasal region of the eye. Due to the general nasal asymmetry, particularly that of the baseplate of the ciliary body, the lens is displaced towards the nasal axis. Therefore, in the kestrel and pigeon the ciliary muscle is closer to the lens nasally, perhaps being able to apply more force in that region. In general, the anterior ciliary muscle fibres are longer and more numerous in the nasal region of the kestrel, chicken and pigeon eye. Murphy *et al.* (1995) reported that the ciliary muscle fibres are shortest in the nasal region of the chicken eye. The reason for this discrepancy between these two studies is not apparent.

The separation of the anterior and posterior muscle fibre groups in the kestrel eye (fig. 8) has also been noted in owls and fish eagles (Brücke, 1846; Krohn in Müller, 1857). However, these authors did not report any circumferential differences of these eyes. In the kestrel the anterior and posterior fibre groups are closer together in the nasal region of the eye, giving it the appearance of a chicken or pigeon ciliary muscle. The separation of the ciliary muscle in raptors makes the fibre groups more distinct, and reflects the basic anatomical divisions described by Crampton (1813), Brücke (1846), and Müller (1857). Lord (1956) was able to divide the raptor ciliary muscle even

further, recognizing a temporal muscle, which probably corresponds to the anterior internal muscle fibre group of this study. The fact that the muscle fibre groups in the kestrel take on more of an unified appearance in the nasal region seems to correspond to the nasal asymmetry in the other parameters studied. However, it is unclear if this asymmetry affects the functioning of the ciliary muscle or is in some way involved in binocularity.

Effect of Contraction

Nicotine sulfate stimulates the muscle fibres with an acetylcholine-like action (Bacq, 1971). With an extreme dosage, as used here (20%), the muscle is depolarized to the point of paralysis (Levy, 1979). The contraction of ciliary muscle can be easily observed or shown quantitatively. The ciliary muscle length of each species shortens by 8-14% with treatment (table I and II). However, some of the individual fibres in the muscle groups demonstrated even more shortening. The anterior fibres in each species contracted the most (10-58%) (table II), possibly reflecting the importance of corneal accommodation (Glasser et al., 1995). However, nicotine sulfate may have easier access to the anterior muscle through the trabecular meshwork. Hooded merganser eyes were treated whole and show the greatest variability in contraction between muscle fibre groups. Removal of the posterior globe aided nicotine sulfate penetration to the muscle, shown by much more consistent results in the chicken, pigeon, and kestrel eyes. These results indicate that the increased contraction of the anterior fibres is not artifactual.

Conclusions

This research agrees with the anatomical description of the avian ciliary muscle as described by Crampton (1813), Müller (1846), and Brücke (1857). In all of the species studied the ciliary muscle can be divided into three main groups (anterior, posterior, and internal) based on their origins and insertions. Although the ciliary muscles of the four species studied are morphologically similar, there are species specific differences that contribute to the relative importance of corneal and lenticular accommodation. Based on the anatomy of the ciliary muscle seen here, chickens and pigeons accommodate by changing the shape of the cornea and lens whereas in kestrels corneal accommodation likely predominates. Lenticular accommodation likely predominates in hooded mergansers. In all species, the anterior fibre group contracted the greatest amount, possibly indicating that the ciliary muscle has a primary role in corneal, not lenticular, accommodation.

Section II

The Functional Anatomy of the Ciliary Muscle in Humans as a Function of Age

Introduction

The human ciliary muscle is composed of smooth muscle fibres that are divided into three muscle fibre types: meridional or longitudinal, radial, and circular. Most of the descriptions of the anatomical structure of the ciliary muscle are from work conducted at the turn of the century. The first description of the ciliary muscle in the literature was apparently made by Eustachius in 1560 (Duke-Elder, 1961). However, its discovery is often attributed to Brücke (1846), whose name is synonymous with the longitudinal fibres. The longitudinal fibres extend along the external edge of the muscle, attaching to the sclera. They originate at the choroid near the ora serrata and insert onto the scleral spur (Wolff, 1968; Hogan *et al.*, 1971). There is some evidence that these fibres are attached by a ciliary tendon that extends through the scleral spur to the corneoscleral meshwork (Rones, 1958; Kupfer, 1962). However, fibres are not seen anterior to the scleral spur (Hogan *et al.*, 1971). Some researchers have described the entire ciliary muscle as inserting onto the scleral spur (Calasans, 1953; Stark, 1988). The attachment of the posterior longitudinal fibres to the choroid region has been described as a direct connection to the choroid (Calasans, 1953), an attachment to the elastic network of Bruch's membrane in the choroid (Rohen, 1964), and a connection to the posterior, peripheral zonules (Stark, 1988). The longitudinal fibres end in muscle stars which can only be seen in teased preparations (Wolff, 1968). The longitudinal fibres are separated by thin layers of connective tissue (Hogan *et al.*, 1971). The radial fibres, located just internal to the longitudinal fibres, are separated by thicker layers of connective tissue (Hogan *et al.*, 1971). The radial fibres have been described as having

attachments to the ciliary processes (Calasans, 1953; Stark, 1988). Müller (1857) is credited with the discovery of the circular fibres and hence they are often referred to as Müller's muscle (Duke-Elder, 1961). The circular fibres are located at the most internal anterior edge of the ciliary muscle and are thought to have a sphincter-like action (Duke-Elder, 1961; Wolff, 1968). Some of these fibres may attach to the iris (Calasans, 1953; Stark, 1988).

The longitudinal fibres are the first to develop embryonically in the ciliary muscle and are clearly visible between the third and fifth month (Hogan *et al.*, 1971; Sellheyer and Spitznas, 1988; Barishak, 1992). The muscle is described as being triangular shaped with the apex at the scleral spur and the base posterior and internal. The radial and circular fibres appear during the seventh month (Barishak, 1992) and are not completely developed at birth (Hogan *et al.*, 1971).

Ciliary Muscle Function

The function of the ciliary muscle is two-fold; accommodation and aqueous outflow. The anterior longitudinal fibres of the ciliary muscle insert onto the scleral spur, the corneal stroma, and the corneoscleral meshwork (Rohen *et al.*, 1981). Contraction of the ciliary muscle results in spreading of the corneoscleral part of the trabecular meshwork and increased aqueous drainage. Therefore, change in aqueous outflow occurs mainly due to contraction of the longitudinal fibres.

The action of the ciliary muscle during accommodation is generally described as a forward movement of the muscle that releases tension on the zonules (Hogan *et al.*, 1971; Kaufman, 1992). The ciliary muscle is described as shortening longitudinally, pulling the choroid forward while the circular fibres draw the ciliary body closer to the lens with a sphincter-like action (Duke-Elder, 1961; Wolff, 1968; and Hogan *et al.*, 1971). This releases tension on the zonules and allows the lens to become thicker. Early investigations using monkeys showed that the relaxed muscle flattens and the contracted muscle moves forward and becomes shorter (Duke- Elder, 1961). However, a recent study of the rhesus monkey ciliary muscle treated with pilocarpine shows the inner apex of the muscle moves forward and the muscle becomes narrower, not thicker (Lütjen-Drecoll *et al.*, 1988b). Schachar and Anderson (1995) and Schachar (1996) have hypothesized, based on pictures of the primate ciliary muscle from other researchers, that the anterior portion of the ciliary muscle curls towards the sclera with contraction. The longitudinal fibres pull the posterior ciliary muscle forward and the circular and radial fibres keep the equatorial zonules taut. This supports Schachar's theory which states that the equatorial zonules are pulling the equator of the lens, causing peripheral flattening during accommodation (Schachar and Anderson, 1995; Schachar, 1996). It also assumes that the zonular fibres extend from the equator of the lens to the far anterior edge of the ciliary muscle, although this feature has not been verified.

There is evidence that the proportion of circular, radial and longitudinal muscle fibres changes with ciliary muscle contraction (Rohen, 1964; Lütjen, 1966). In primates, the surface area of longitudinal, radial, and circular fibres from the ciliary

muscle was measured from eyes treated with a variety of drugs (Lütjen, 1966). It was concluded that the percentage of circular fibres increases with muscle contraction, while the proportion of longitudinal muscle fibres decreases. The same shift of longitudinal to radial fibres with contraction has been reported in another mammal, the raccoon (Rohen et al., 1989). This would support the reticulum theory of the arrangement of fibres (Rohen, 1964), since contraction of the muscle appears to result in a rearrangement of the fibre system. However, in the Lütjen study (1966), it is unclear if muscles were classified by the treatment or only by the appearance of the muscle. If the appearance of the muscle is used to classify the strength of contraction, it is erroneous to assume that the strongest contraction results in the largest proportion of circular fibres without taking into account drug concentration, etc.

Rohen (1964) proposed that the ciliary muscle fibre groups should not be labeled individually. Rather, he proposed that all the fibres in the ciliary muscle are interconnected to form a reticulum. According to Rohen, the longitudinal fibres cross at very acute angles, whereas the radial fibres cross at wider angles and the circular fibres at still wider angles. The fibres are interwoven with some longitudinal fibres angling enough to become circular fibres. Calasans (1953) described the fibre types as being arranged in V-shaped pairs so that the base of the V for all fibres is located at the scleral spur and the branches of the V are crossing at wider angles for the longitudinal, radial and circular fibres, respectively. The muscle fibre branching has also been described as Y-shaped instead of V-shaped (Nishida, 1986). This arrangement of fibres supports the view that the muscle acts as a synctium to produce movement of the ciliary body.

However, experimental evidence indicates that the ciliary muscle may be able to disassociate its outflow and accommodative functions. In cynomolgus monkeys treated with small doses of pilocarpine, little or no effect was seen on refraction while large changes were detected in uveo-scleral flow (Bill, 1967). The investigator concluded that low concentrations of pilocarpine only affect longitudinal fibres which have little effect on refraction. Erickson-Lamy and Schroeder (1990) have shown that aceclidine produces two dioptres of accommodation in cynomolgus monkeys while pilocarpine produces 19 dioptres. These same drugs produced a similar two fold increase in facility of outflow. This again suggests the dual function of the ciliary muscle and its ability to disassociate these functions. Flügel et al. (1990) have demonstrated a modest difference in the enzyme histochemistry between the tips of longitudinal fibres and the radial and circular fibres in primate ciliary muscles. These differences may indicate that the tips of the longitudinal fibres are responding faster to innervation, leading the rest of the muscle forward and inward (Flügel et al, 1990).

There also is some evidence that the ciliary muscle is composed of functional units that contract independently. When the parasympathetic motors roots to the ciliary muscle were partially sectioned in cats, the muscle was seen to contract only in certain areas (Ripps et al. 1962). In vervet monkeys, the ciliary muscle was injected with small amounts of pilocarpine (Bárány and Rohen, 1965). The excitation from this treatment did not spread very far into the surrounding muscle tissue, indicating that the ciliary muscle acts as a multi-unit muscle with localized contraction and relaxation. Therefore, it is unclear whether the ciliary muscle acts as a single- or multi-unit. The ciliary muscle may be capable of localized contraction and relaxation, although, it is not clear from

these experiments whether the different fibre types can act independently. Anatomical studies of the ciliary muscle have not shown morphological evidence for the ciliary muscle acting in functional units.

Ciliary Muscle Aging

Presbyopia is a universal aging condition which results in an individual's inability to accommodate for near objects (Kleinstein, 1987). Most individuals begin to notice the loss of accommodation at 40-50 years of age with a total loss at approximately 51 years (Charman, 1989). Presbyopia has been attributed to "sclerosis" or hardening of the lens (Fisher, 1971; Pau and Krantz, 1991). Other factors which may contribute to presbyopia include changes in water content of the lens (Pierscionek, 1989), a change in the crystalline content of the lens (Coghlan and Augusteyn, 1977; Pierscionek and Augusteyn, 1988), and the forward migration of the zonular fibres to the anterior face of the lens (Weale, 1962; Farnsworth and Shyne, 1979). While presbyopia is probably a multi-factorial condition, this study will concentrate on the role of the ciliary muscle.

The ciliary muscle has an important function: to initiate accommodation. Thus, a loss of the ability to accommodation necessitates a closer look at how the ciliary muscle may contribute to this condition. The three issues to address are: a) morphological changes in the ciliary muscle with age, b) functional changes in the ciliary muscle with age, and c) the contribution of these changes to presbyopia.

Morphological Changes in the Ciliary Muscle with Age

An increase in intramuscular connective tissue with age in the human ciliary muscle has been documented in the literature (Stieve, 1949 in Duke-Elder, 1961; Tamm et al., 1992b). The increase in connective tissue appears to be concentrated in the reticular or radial region and not the area of longitudinal fibres (Tamm et al., 1992b). In rhesus monkeys there is only a slight increase in the amount of connective tissue within the ciliary muscle with age, although the amount in the ground plate increases (Lütjen-Drecoll et al., 1988 a and b). The human ciliary muscle shortens in length with age, while the width shows no significant change (Tamm et al., 1992b). In a monkey eye treated with atropine, the ciliary muscle becomes shorter with a smaller area while in one treated with pilocarpine the muscle becomes shorter and narrower with age (Lütjen-Drecoll, 1988b). Tamm et al. (1992b) also documented an age related change in the total area of longitudinal, radial and circular fibres in the human ciliary muscle. The area of longitudinal and radial portions decreases with age while the circular portions increases. Study of the ultrastructure of the ciliary muscle cells in young, old, and prostaglandin $F_{2\alpha}$ treated monkey eyes shows that age changes are similar to prostaglandin treated eyes in which the muscle cells lose their connections to extracellular fibrils in basal lamina (Tamm et al., 1990). The posterior attachment of the ciliary muscle in monkeys consists of elastic tendons which have been reported to thicken with age (Tamm et al., 1991).

Functional Changes in the Ciliary Muscle with Age

Are these morphological changes causing functional changes in the ciliary muscle? Studies of the human ciliary muscle have shown no decrease in muscle activity with age (using impedence cyclography: Swegmark,1969; using indirect lens measurements: Fisher, 1977; and using magnetic resonance: Strenk and Semmlow, 1995). Other investigators have shown an age-related loss of ciliary muscle mobility in monkeys (Bito *et al.*, 1987a and b; Lutjen-Drecoll *et al.*, 1988b; Neider *et al*, 1990). It has been concluded that the loss of posterior attachment elasticity with age accounts for the loss of ciliary muscle mobility and therefore contributes to presbyopia (Tamm *et al.*, 1992a; Poyer *et al*, 1993).

The Role of the Ciliary Muscle in Presbyopia

As noted earlier, there are many theories as to the causes of presbyopia ranging from lenticular theories to extralenticular theories (see Atchison, 1995 for a review). Some of these propose that ciliary muscle contraction remains constant throughout life (Hess-Gullstrand theory and Fincham theory) (Atchison, 1995). Others hypothesize that the ciliary muscle weakens with age (Duane, 1925). The rhesus monkey is a suggested animal model for presbyopia because of the decrease in accommodative amplitude with age (Bito *et al*, 1982). However the mechanism underlying presbyopia may not be the same in human and monkeys. The investigations cited above show that ciliary muscle activity appears to remain unchanged in humans, but declines with age in primates. Another example of the differences between species is that many of the changes associated with age in the human ciliary muscle are similar to the changes in

configuration of the muscle with contraction (Tamm *et al.*, 1992b). However, the

opposite is true in monkeys, where the age changes resemble the configuration of the

young relaxed ciliary muscle (Lütjen-Drecoll *et al*, 1988 a and b). While the structure

of the ciliary body in humans and primates is similar, the causes of presbyopia may not

be the same and generalizations may not be accurate.

It is important to note that in spite of the importance of accommodation in

everyday life and in spite of an enormous literature on the psychophysical study of

human accommodation, there has been a scarcity of anatomical study of the muscle

responsible. The few studies on the human ciliary muscle do not examine functional

morphology. Thus, the current study is the first study of the human ciliary muscle in

which contraction and relaxation of the muscle is considered as a function of age. One

other study has examined the human ciliary muscle after pilocarpine- and atropine-

treatment, but the methods are not described (Rohen, 1964). Other studies of the

functional anatomy of the ciliary muscle with contraction have been carried out on

primates (Lütjen, 1966; Lütjen-Drecoll *et al.*, 1988b). Studies on age-related anatomical

changes of the human ciliary muscle have not treated the eyes to induce contraction

(Tamm *et al.*, 1992b). The non-anatomical studies on the changes in ciliary muscle

mobility with age have a limited age range (Swegmark, 1969; Fisher, 1977 Stark and

Semmlow, 1995). This study examines the morphological changes of the human ciliary

muscle after treatment with pilocarpine and atropine in 15 pairs of eyes ranging from 0

to 107 years. The differences between the contracted and relaxed muscle was studied to

determine the function of the human ciliary muscle in accommodation. The ciliary

muscle was also analyzed for morphological change in the response of the ciliary

muscle with age.

Methods

Sixteen pairs of human eyes, ranging in age from one day to 107 years old, were obtained from the Eye Bank of Canada, Ontario Division. Eyes were enucleated an average of 5 hours after death of the donor (minimum: 2 hours, maximum: 13 hours). The eyes were placed on saline-soaked gauze in glass bottles and shipped on ice in thermoses. All eyes received appeared healthy with clear lenses. The majority of eyes were processed between 24 and 48 hours after death with two pairs being processed after 72 and 96 hours after death. No anatomical differences were seen in eyes processed at different times post-mortem at the magnification used.

The following ocular dimensions of each eye were measured to the nearest 0.05mm using vernier calipers: axial length, the equatorial diameter, and the pupil diameter. The optic nerve of each eye was carefully removed using scissors and the eye was placed cornea-side down in a dissection dish. The right eye of the pair was left untreated in ages: 59, 62, 79, and 107. The right eyes of the remaining ages were treated with 20% atropine sulfate (Sigma) in phosphate buffer (pH 8), while the left eye of each pair was treated with 5% pilocarpine hydrochloride (Sigma) in phosphate buffer (pH 6). Both concentrations are considered maximal (approximately 5 times the concentration used *in vivo*)(O'Connor Davies *et al.*, 1989) in order to induce the greatest change in ciliary muscle response. Each eye was bathed with the drug several times over the next 30 minutes. Measurements of the pupil diameters were made after treatment to assess the reactivity of the eye to the drug. The eyes were then placed in 2% glutaraldehyde in saline (pH 7.4) along with the drug (see Tamm *et al.*, 1992a). The

central area of each cornea was removed after 12-24 hours of fixation to facilitate

penetration of the fixative. Each pair of eyes was fixed for a minimum of 7 days while

being stored at 0° C. A fixation time of greater then 14 days produced optimal staining

and preservation of tissue.

After fixation, the posterior portion of the globe was carefully removed

approximately 3mm posterior to the ora serrata and each anterior portion was embedded

whole in JB-4 methacrylate plastic (J.B. EM Services). The embedded anterior portion

was then divided into eight pie-shaped wedges, isolating the ciliary body and a portion

of the lens. Three random wedges were embedded again in JB-4 for sectioning with a

Reichert microtome at 7-9 microns.

The orientation of the eyes was only marked in eyes from the 34 and 65 year old

donors which were used to study asymmetric differences in the anatomy of the ciliary

muscle. In these two pairs of eyes orientation was determined by examining the

position of the optic nerve and the shape of the pupil in the iris (the pupil is displaced

nasally, Hogan *et al.*, 1971). Eyes from the 34 and 65 year old donors were not

embedded whole but divided into wedges referred to as superior (S), superior nasal

(SN), nasal (N), nasal inferior (NI), inferior (I), inferior temporal (IT), temporal (T), and

superior temporal (ST). These wedges were then individually embedded in JB-4 and

sectioned as above.

Approximately 28 sections per wedge or 84 sections per eye were obtained for

all eyes. For the eyes in which asymmetry was studied, 224 sections per eye were

obtained. The sections were stained with a modified trichrome stain on a slide warmer

(56°C). All slides were treated with the following protocol which was developed during

this study: celestine blue (Bancroft and Stevens, 1991) for 5 min, rinsed with distilled

water, Mayer's hematoxylin (Bancroft and Stevens, 1991) for 10 min, "blued" in tap

water, acid fuschin (Humason, 1962) for 9 min., rinsed in distilled water, and fast green

(in a phosphate buffer, pH 7.4)(Humason, 1962) for 7 minutes, and rinsed with distilled

water, then allowed to air dry. The slides were coverslipped with permount and

examined using bright field microscopy on a Nikon (Labophot-2A) light microscope.

All eyes were used for quantitative analysis, expect the eyes from the 62 year old

donor which were not analyzed due to processing artifacts. Measurements of ciliary

muscle length, width, the distance from the widest portion of the muscle to the most

anterior point (anterior length) and the distance from the widest portion of the muscle to

the most posterior point (posterior length) were made using a microscope with a

drawing tube which projected the image onto a computer monitor (Samsung

SyncMaster 17glsi). The error caused by projecting the image onto a curved monitor

screen was negligible, since the measurement error was always greater (see below for

values). Six sections per eye were measured in 15 pairs of eyes for a total analysis of

180 sections. All slides were masked and randomized so that the age and treatment

were unknown to the investigator. A histology tracing program was used to draw

around the perimeter of the muscle at 40X magnification. The total length measurement

was taken by drawing a line parallel to the scleral surface from the most anterior and

posterior attachment points (fig. 1). The width measurement was made by drawing a

line perpendicular to the length measurement at the widest part of the muscle (fig. 1).

The distance from the anterior muscle attachment to the widest point was determined by

drawing a line from the anterior-most point to the intersection of the line denoting the

Figure 1: A tracing of the ciliary muscle showing the measurements taken for this study.
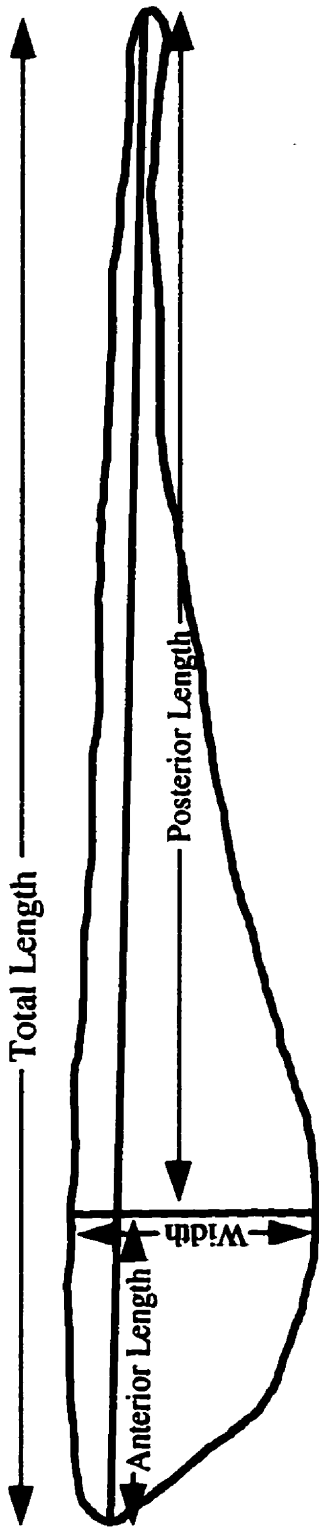
widest part of the muscle (anterior length) (fig. 1). The posterior length was determined by subtracting the anterior length from the overall length measurement. The measurement error (found by taking repeated measurements of a single section) was calculated as ±0.36 mm for length, ±0.004 mm for width and ±0.022 mm for anterior length. The greater measurement error for total length is due to the fact that the entire muscle could not be viewed at one time. Thus the microscope and computer image had to be scrolled in order to determine total length of the muscle.

The area of each muscle fibre type was also measured in 15 pairs of eyes using six sections from each eye. The drawing tube was used to project the image of the ciliary muscle onto a computer monitor at a magnification of 100X. Higher magnifications were used as needed in order to see the shape of the muscle nuclei which were used to classify the orientation of muscle fibres. The ciliary muscle was sectioned longitudinally, thus the circular fibres are seen in cross-section. The muscle cell appears circular with a round nuclei near the center (fig. 2a). Radial fibre muscle cells were sectioned obliquely so that the fibres appear irregular-shaped with oval nuclei (fig. 2b). The longitudinal fibres were sectioned along the long-axis of the cell and thus appear narrow and elongated. The nuclei also have this elongated appearance (fig. 2c). The boundary between the circular and radial fibres was the most difficult to determine due to a number of cells with round nuclei and elongated cell bodies or oval nuclei with round cell bodies. Even though care was taken in embedding to ensure precise orientation, it is unlikely that all fibres were in the optimal plane of section for correct determination of orientation. Fibres were first judged by nuclei shape and then by the shape and apparent direction of the muscle fibre. Again all slides were masked and

Figure 2: Micrographs of the three types of muscle fibres orientations in the ciliary

muscle. (A) The circular fibres are characterized by round nuclei and fairly round cell

bodies. (B) The radial fibres contain oval shaped nuclei and the muscle cell bodies are

arranged at oblique angles. (C) The longitudinal fibres contain elongated nuclei and the

cell bodies are also sectioned along the long axis.

0.05 mm

randomized so that the age and treatment were not known to the investigator. The boundary of each fibre type was drawn and the area automatically calculated by the program. The areas were then calculated as a percent of the total area for comparison across ages. The measurement error for this measurement is ±1.5% based on five repeated measurements from one section.

The percentage of muscle compared to connective tissue in the ciliary muscle was calculated using the histology tracing program. Six sections from each eye were analyzed in the same 15 pairs in which fibre direction was analyzed. Each muscle was photographed with slide film using a Nikon FX-35WA camera attached to a Nikon Labophot Microscope at 12.5X magnification. Many muscles had to be photographed as a montage. The slides were then scanned into a computer using a slide scanner and commercial graphics program (Abode Photoshop). Using the graphics program, the ciliary muscle was cut out of each picture and pasted onto a new file. A montage of the ciliary muscle was compiled when necessary. The image of the ciliary muscle was then copied into the histology tracing program where the threshold (red or green coloration) that most closely matched the muscle fibre staining was determined. The program then shades all pixels within that threshold, allowing a determination to be made as to whether only the muscle and not connective tissue was being colorized. The program then reports the percentage of muscle tissue versus connective tissue. The measurement error for calculating the percentage of muscle is ± 0.76%, on the basis of a trial of five repeated measurments of the same muscle.

A statistical analysis was performed on the above data from fifteen pairs of eyes to determine if there are differences between the relaxed and contracted eyes, to

examine changes in the muscle with age and to determine if the muscle is responding

the same to treatment across age (i.e. are there functional changes with age?). The data

were arranged roughly into decades as follows:eyes from the 0, 0.67, and 4 year old

donors into the 0-4 age group; eyes from the 34, 42, and 43 year old donors into the 30-

50 age group; eyes from the 54, 59, and 65 year old donors into the 50-70 age group;

eyes from the 75, 78, and 79 year old donors into the 70-80 age group; and eyes from

the 83, 85, and 107 year old donors into the 80-110 age group. A three-way repeated

ANOVA was performed on wedge versus eye versus decade for each above

measurement ($p < 0.05$). Linear contrasts (Systat) were used to determine differences

between specific age groups in the main effect of age ($p < 0.025$) (Howell, 1992).

Nasal-temporal asymmetries were analyzed in the eyes from 34 and 65 year old

donors. Length, width, anterior length, and posterior length measurements were taken

on 5 sections per wedge or 40 sections per eye. The areas of the circular, radial, and

longitudinal fibres in the ciliary muscle were analyzed using 3 sections per wedge or 24

sections per eye, as in the analysis on the proportion of muscle vs. connective tissue.

Over 350 sections were examined for the asymmetry analysis. A three-way repeated

measures ANOVA was performed to test for differences between the wedges, which

might indicate asymmetries around the circumference of the eye; for differences in the

atropine and pilocarpine treated eyes, which might indicate asymmetric contraction of

the ciliary muscle; and for differences between the ages. Due to uncertainty in

orientation, the superior nasal, nasal, and nasal inferior wedges were grouped together

as the nasal region as were the superior temporal, temporal, and inferior temporal

wedges for the temporal region. The analysis was only performed on the difference

between the nasal and temporal regions, since this is where the main asymmetry of the ciliary body has been reported (Hogan *et al.*, 1971). Significant interactions were analyzed using simple effects ($p < 0.05$) (Howell, 1992).

Results

General Anatomical Features

In eyes from donors less than one year (1 day old and 8 month old) the ciliary processes are located far anterior along the ciliary body (fig. 3). The processes are clumped behind the iris, with most of the ciliary processes anterior to the ciliary muscle, although a few processes are seen to extend approximately 1/8 to 1/4 down the anterior edge of the ciliary muscle. After age 34, the ciliary processes are uniformly spaced along the internal edge of the ciliary body for approximately 1/3 to 1/2 the distance of the ciliary muscle (fig. 4).

Portions of the zonules are visible in most eyes, although no single zonule could be traced from insertion to origin. The zonules attach on the posterior, anterior, and equatorial margins of the lens capsule and course back towards the ciliary processes. The zonules originate along the ciliary muscle at the ciliary epithelium, farther posterior than the ciliary muscle. Two sets of zonular fibres, which appear to cross at the ciliary processes, could be identified. The zonules originate on the ciliary epithelium. One set courses forward in the eye and the other set backwards (fig. 5). These fibres may be analogous to the description of the "tension fibre" system (Rohen and Rentsch, 1969; Rohen, 1979). The tension fibres anchor the main fibres, allowing them to curve along the margin of the ciliary body.

Three muscle fibre orientations, circular, radial and longitudinal, are visible at every age. The region of longitudinal fibres is the greatest (41-69%), followed by radial fibres (25-47%) and circular fibres (4-24%). The circular fibres are characterized by

Figure 3: The ciliary muscle from a 0 year old donor (1 day old) treated with atropine

(A) and pilocarpine (B). In young eyes, the ciliary processes (CP) are located far

anterior to the ciliary body. The muscle fibres are packed tightly together with little

connective tissue. The area of longitudinal (L) fibres is easily identifiable, however, the

circular fibres (C) are intermixed with the radial fibres (R) making it difficult to locate

specific areas of exclusively circular or radial fibres. The ciliary muscle is located

internal to the sclera (S) and the root of the iris can be seen (I).

Figure 4: The ciliary muscle from a 43 year old donor treated with atropine (A) and

pilocarpine (B). The three fibre orientations of the ciliary muscle are visible: the

circular fibres (C) are located along the anterior and internal edge; the longitudinal

fibres are elongated (L) and found along the scleral surface; and the radial fibres (R) are

oblique fibres that are located between the circular and longitudinal fibres. Eyes from

this age group (30-50) reacted opposite to the other groups in that the ciliary muscle

lengthened, not shortened with treatment. The edges of the ciliary processes (CP) can

be seen internal to the muscle for about 2/3 of the muscle length. Also note that the

internal, anterior edge of the ciliary muscle did not move forward with contraction,

although the fibres along the internal edge become more prominent.

0.20 mm A

0.20 mm B

CP
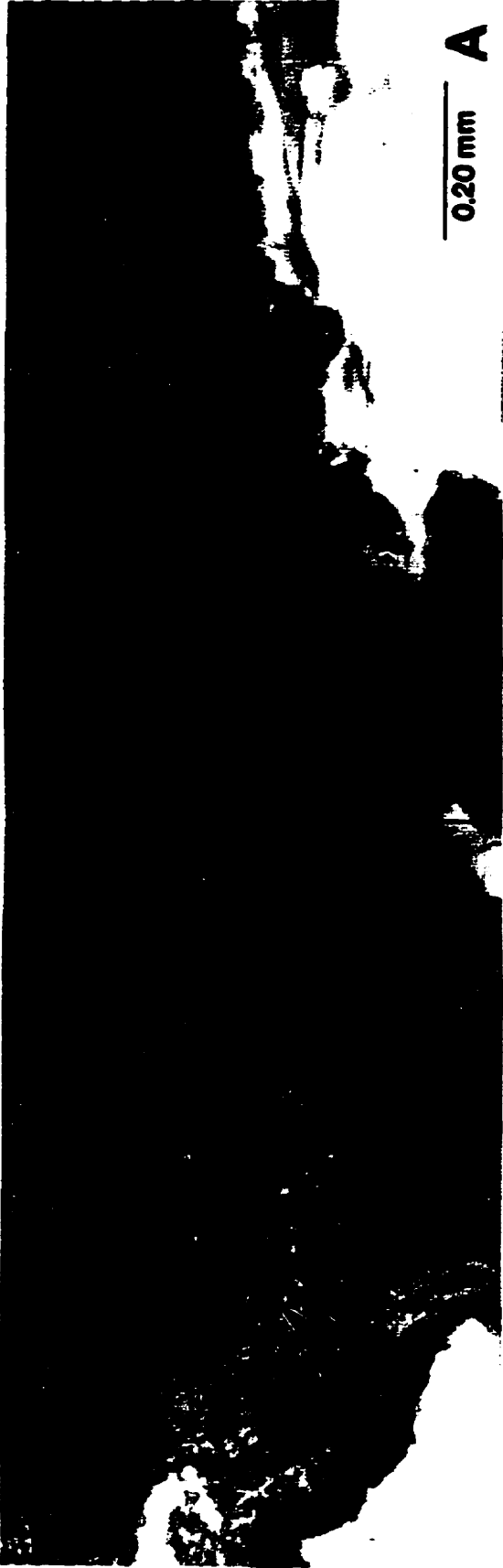
Figure 5: A micrograph at high magnification, showing the tension fibres (T) of the

zonular system attaching to the internal surface of the ciliary epithelium (E) under the

ciliary muscle (M). The crossing of the tension fibres can be seen as the fibres course

both anteriorly and posteriorly to attach to the main zonular system (Z).

0.05 mm

round nuclei that are positioned at the centre of the muscle cell (fig. 2). In the eyes less than 1 year, the circular fibres are present, but do not form bundles. Instead the circular fibres are intermixed with the radial fibres near the centre of the muscle (fig. 3). In older eyes the circular fibres form bundles near the anterior and anterior internal edge, extending further along the internal edge with age (fig. 4 and 6). The radial fibres contain oval nuclei (fig. 2) and course obliquely across the ciliary muscle from near the scleral spur to the internal edge of the muscle. Intermuscular connective tissue is more abundant among the radial and circular fibres (fig. 4). The longitudinal fibres are characterized by the appearance of elongated nuclei and thin muscle fibres (fig. 2) that are arranged along the scleral edge of the ciliary muscle, from the scleral spur to the choroid. The longitudinal fibres are the only fibre orientation that appeared to directly originate at the scleral spur. The circular and radial fibres are the most difficult to distinguish. Fibres that have round nuclei, appear to be cut in cross-section, and lack an obvious direction, are considered circular. Any fibres that appear to be sectioned obliquely, along the longitudinal axis, are considered radial.

The young ciliary muscle appears to consist of individual fibres uniformly distributed with little connective tissue (fig. 3). As the muscle ages, the muscle fibres appear to form bundles with greater amounts of connective tissue between these bundles, particularly in the radial and circular areas (fig. 4 and 6). Perhaps due to this, the orientation of the fibre types in the older eyes is much more prominent.

Figure 6: The ciliary muscle from an 85 year old donor treated with atropine (A) and

pilocarpine (B). When compared with the ciliary muscle from the 43 year old donor, the

widening and forward movement of the ciliary muscle with age is clearly evident. The

amount of connective tissue in the ciliary muscle increases with age, which makes the

three fibre types, circular (C), radial (R), and longitudinal (L), even more identifiable.

A

0.20 mm

B

0.20 mm

Treatment Effects

One eye of each pair was treated with atropine, a parasympatholytic agent. Atropine blocks parasymathetic activity by competing with acetylcholine at the effector cell, thus preventing depolarization (Thompson, 1987). The opposite eye was treated with pilocarpine, a parasympathomimetic which has a similar configuration to acetylcholine, causing depolarization of the effector cell (Thompson, 1987). For this study atropine is considered to induce relaxation of the ciliary muscle, similar to that occurring in the unaccommodated state and pilocarpine is considered to induce contraction of the ciliary muscle, reflecting the accommodated state (van Alphen *et al.*, 1962).

Measurements of pupil diameter before and after treatment were used to verify that the drug penetrated the eye. In all pairs, the pupil of the atropine-treated eye is larger in diameter than the pilocarpine-treated eye, indicating that the tissue was reacting to the drug.

The appearance of the individual muscle fibres in the ciliary muscle is not visibly different when the atropine- and pilocarpine-treated eyes are compared. This is probably due to the nature of smooth muscle fibres. The shape of the individual fibres is difficult to discern since the fibres form bundles and also run at various angles to the plane of section. However, changes in the overall shape of the ciliary muscle with treatment are evident and quantifiable.

Ciliary muscle dimensions, relaxed and contracted

Changes in total length of the ciliary muscle are not consistent with age (repeated ANOVA $F_{(4, 25)}$ = 3.32, $p < 0.05$). The ciliary muscle shortens with pilocarpine treatment in all age groups (8-20 %) except in the 30-50 year age group in which the length of the ciliary muscle increases (8 %) with contraction (fig. 7). In this age group, two out of the three pairs of eyes (from 34, 43 year old donors) lengthened with pilocarpine treatment. (Table I) Overall the contracted muscles are shorter than relaxed muscles ($\bar{x}$ = 3.74 vs. $\bar{x}$ = 4.08 mm). All age groups showed some difference in length with treatment, indicating no age related loss of the ability of the muscle to contract. In fact, the largest difference between the relaxed and contracted states occurred in eyes from an 85 year old donor (31%) (Table I). The length of the ciliary muscle increases significantly between the 0-4 and 30-50 year age groups ($\bar{x}$ = 3.13 and $\bar{x}$ = 4.54 mm, respectively) and then shortens significantly in the next age group from 50-70 years ($\bar{x}$ = 3.88 mm). The length of the muscle then remains constant throughout the remaining age groups. (fig. 8a)

The difference in the width of the ciliary muscle with pharmacological treatment is not consistent with age. The muscle width decreases in all muscle fibre groups (5-14%), except the eyes from the 30-50 year old donors in which the muscle widens with contraction (10%) (see fig. 9). In this group, two out of the three pairs of eyes showed muscle width increases with contraction (from the 34 and 42 year old donors)(Table I). Summing all the data together for the treatment effect indicates that the muscle significantly narrows with contraction (atropine-treated eye: $\bar{x}$ = 0.52 mm; pilocarpine-treated eye: $\bar{x}$ = 0.5 mm). All age groups showed differences in width between

Figure 7: Total length of the ciliary muscle across age
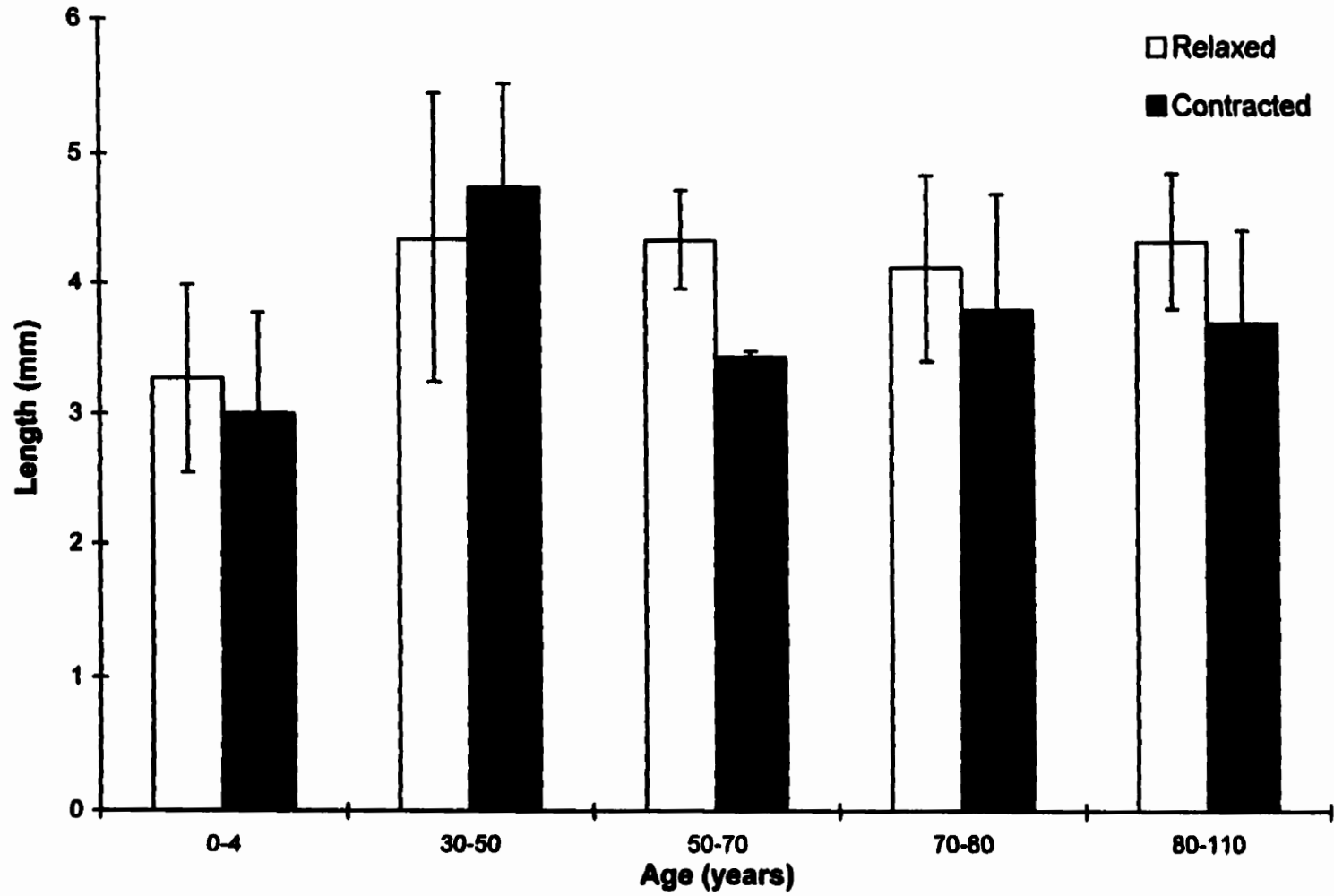
Table I: The total length, width, anterior length, and posterior length measurements for each age

Age (years)	Total Length		Width		Anterior Length		Posterior Length	
	Atropine	Pilo.	Atropine	Pilo.	Atropine	Pilo.	Atropine	Pilo.
0	2.45 ±0.26	2.13 ±0.11	0.43 ±0.03	0.39 ±0.05	0.61 ±0.10	0.50 ±0.05	1.84 ±0.18	1.63 ±0.12
0.67	3.55 ±1.87	3.29 ±0.75	0.46 ±0.04	0.39 ±0.02	0.67 ±0.19	0.63 ±0.19	3.03 ±1.72	2.66 ±0.66
4	3.80 ±0.74	3.58 ±0.68	0.34 ±0.02	0.27 ±0.08	0.55 ±0.16	0.69 ±0.18	3.25 ±0.73	2.89 ±0.73
34	4.13 ±0.28	5.56 ±0.84	0.56 ±0.09	0.67 ±0.03	0.72 ±0.12	0.66 ±0.21	3.41 ±0.23	4.90 ±0.75
42	5.54 ±0.51	4.66 ±0.38	0.48 ±0.02	0.54 ±0.05	1.09 ±0.22	0.70 ±0.15	4.45 ±0.57	3.95 ±0.46
43	3.36 ±0.60	4.01 ±1.11	0.46 ±0.03	0.45 ±0.07	0.53 ±0.26	0.71 ±0.16	2.83 ±0.46	3.29 ±1.17
54	4.21 ±0.47	3.40 ±0.48	0.47 ±0.03	0.41 ±0.02	0.69 ±0.05	0.71 ±0.29	3.53 ±0.48	2.69 ±0.24
59	4.77 ±1.35	3.49 ±0.72	0.64 ±0.07	0.56 ±0.06	0.86 ±0.27	0.52 ±0.19	3.91 ±1.17	2.96 ±0.62
62	4.57 ±0.95		0.73 ±0.03		0.57 ±0.18		4.00 ±0.95	
65	4.03 ±2.04	3.41 ±0.30	0.47 ±0.05	0.52 ±0.02	0.47 ±0.08	0.36 ±0.09	3.56 ±1.98	3.04 ±0.27
75	4.29 ±0.77	4.07 ±0.86	0.55 ±0.06	0.48 ±0.04	0.53 ±0.14	0.54 ±0.15	3.77 ±0.67	3.54 ±0.78
78	3.33 ±0.26	2.78 ±0.41	0.58 ±0.10	0.45 ±0.04	0.40 ±0.10	0.51 ±0.12	2.93 ±0.25	2.27 ±0.41
79	4.74 ±0.70	4.52 ±1.83	0.50 ±0.03	0.55 ±0.03	0.55 ±0.12	0.57 ±0.42	4.19 ±0.76	3.96 ±1.46
83	3.72 ±0.31	3.32 ±0.40	0.53 ±0.03	0.48 ±0.05	0.72 ±0.08	0.54 ±0.17	3.01 ±0.27	2.78 ±0.47
85	4.67 ±0.45	3.23 ±0.68	0.60 ±0.06	0.61 ±0.04	0.32 ±0.06	0.37 ±0.11	4.35 ±0.44	2.86 ±0.61
107	4.60 ±1.26	4.53 ±0.82	0.75 ±0.06	0.70 ±0.05	0.44 ±0.10	0.44 ±0.10	4.16 ±1.19	4.09 ±0.74

Figure 8a: Total length and posterior length of the ciliary muscle versus age

Figure 8b: Width and anterior length of the ciliary muscle versus age
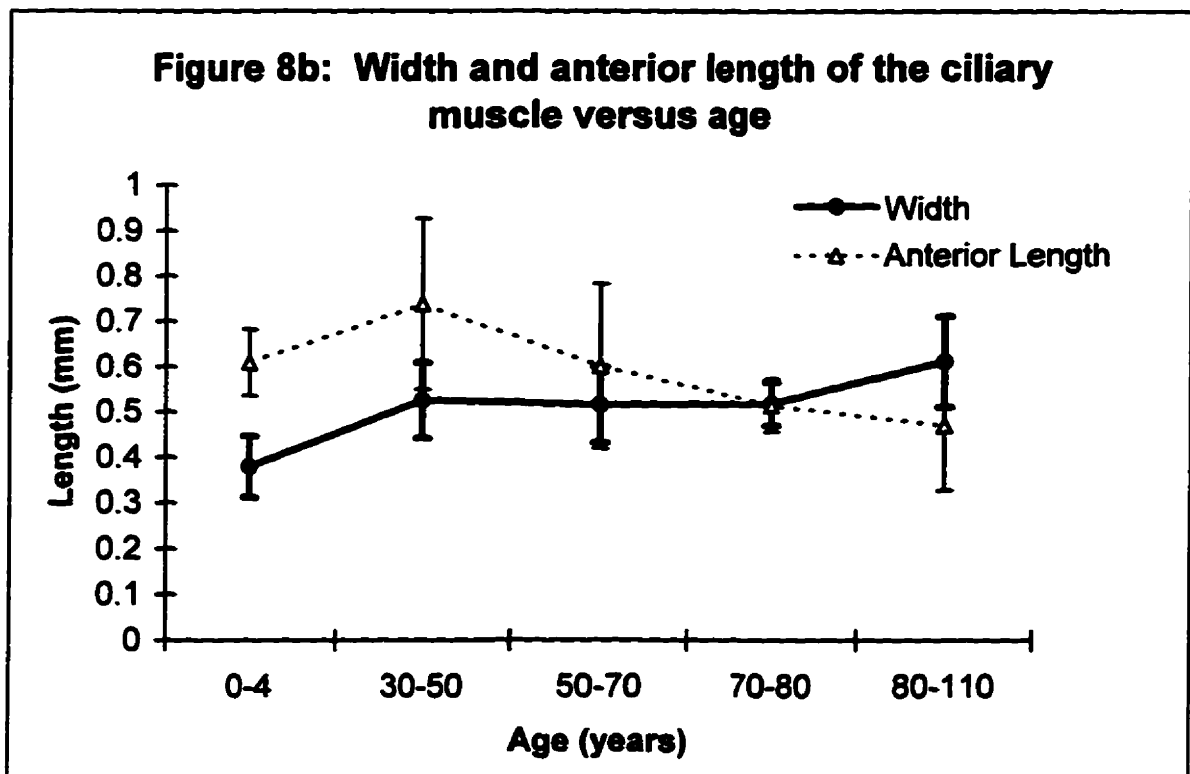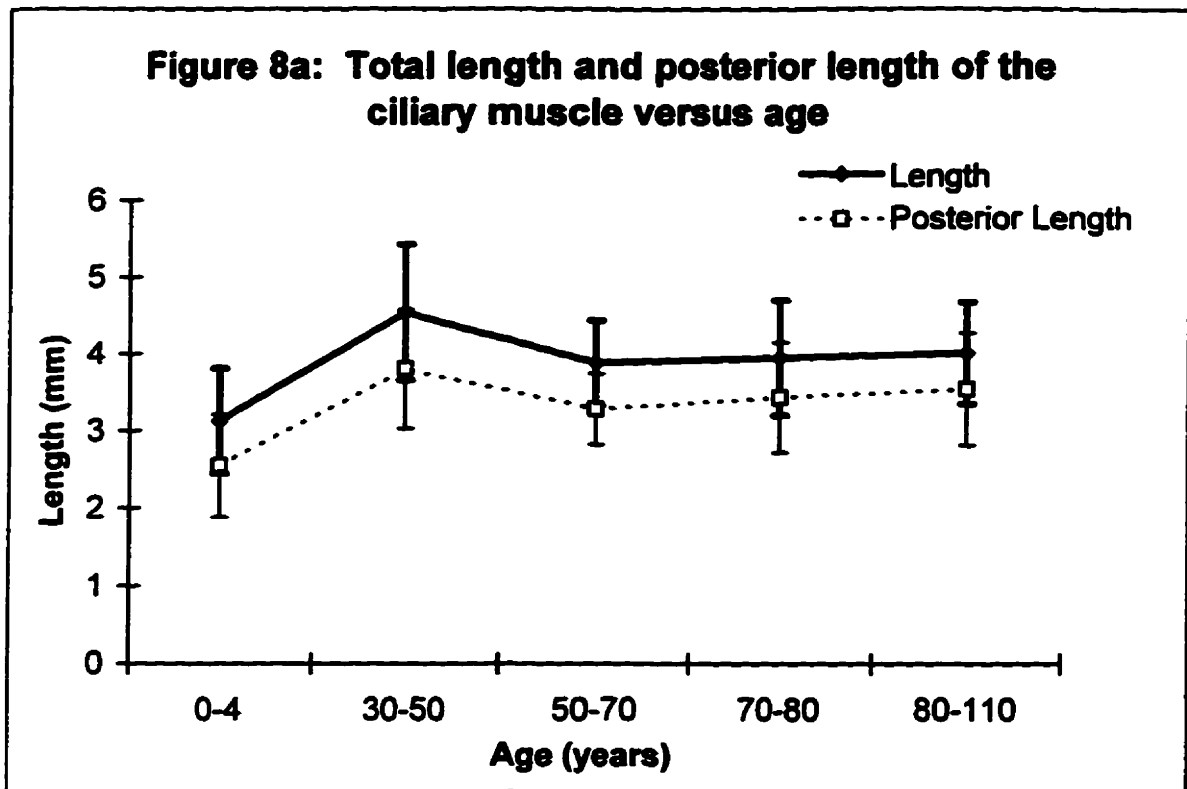
Figure 9: The width of the ciliary muscle across age
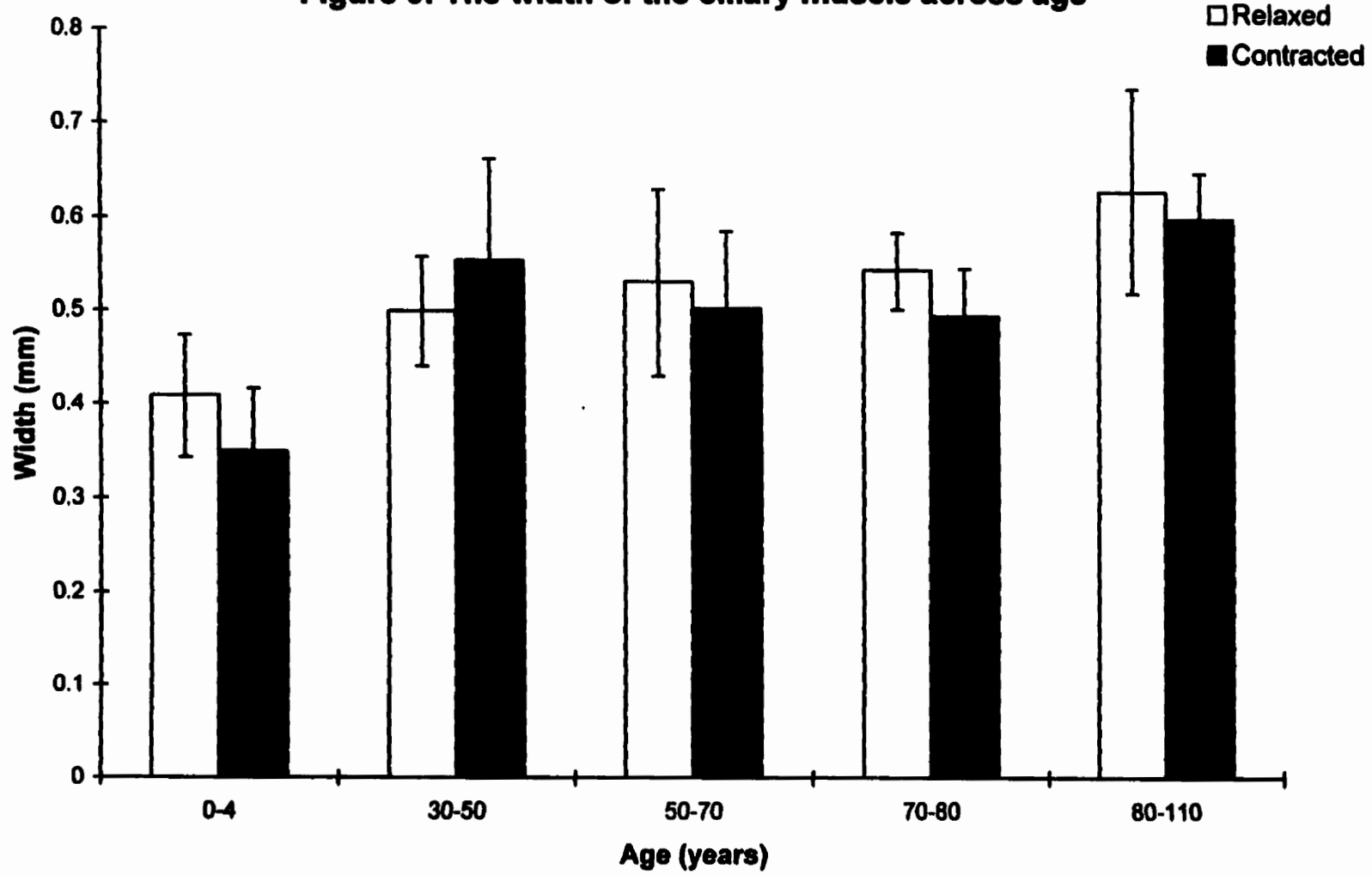
the relaxed and contracted states, again indicating no loss of the ability of the muscle to contract. With age, the width of the muscle increases significantly (Δ= 38%)(fig. 8b).

The anterior length of the ciliary muscle, from the widest portion to the most anterior point, is not significantly different between the relaxed and contracted states and there is also no relationship between changes with contraction and age. This measurement reflects the position of the ciliary processes in the eye in relation to the lens. If the anterior length shortens, the ciliary body is wider more anteriorly and thus the ciliary processes are going to move forward as well. Although no significant changes are detectable with contraction, all age groups reacted to the treatment, again indicating that the ciliary muscle is able to contract throughout life. However, there is a significant shortening of anterior length with age, when summing the treatment groups. The muscle increases insignificantly in anterior length between the first two age groups (17%) and then shortens significantly by 36% (fig. 8b).

The posterior length of the ciliary muscle, from the widest portion to the most posterior attachment point, follows the same trend as the total length measurements. This measurement along with the anterior length more precisely describe the shape of the ciliary muscle. The posterior length provides an indication of the relative distance of the ciliary processes from the ora serrata. The difference in posterior length between the pilocarpine and atropine treated eyes depends on age [$F(4,25) = 3.79$, $p<0.05$]. The posterior length shortens with contraction in all age groups (10-21%), except in the 30-50 year old donors, where the muscle increases in length by 12% (fig. 10). Overall the posterior length shortens significantly with muscle contraction (relaxed: $\bar{x} = 3.48$ vs. contracted: $\bar{x} = 3.17$ mm). All ages reacted to the treatment, again indicating no loss of

Figure 10: Posterior length of the ciliary muscle across age
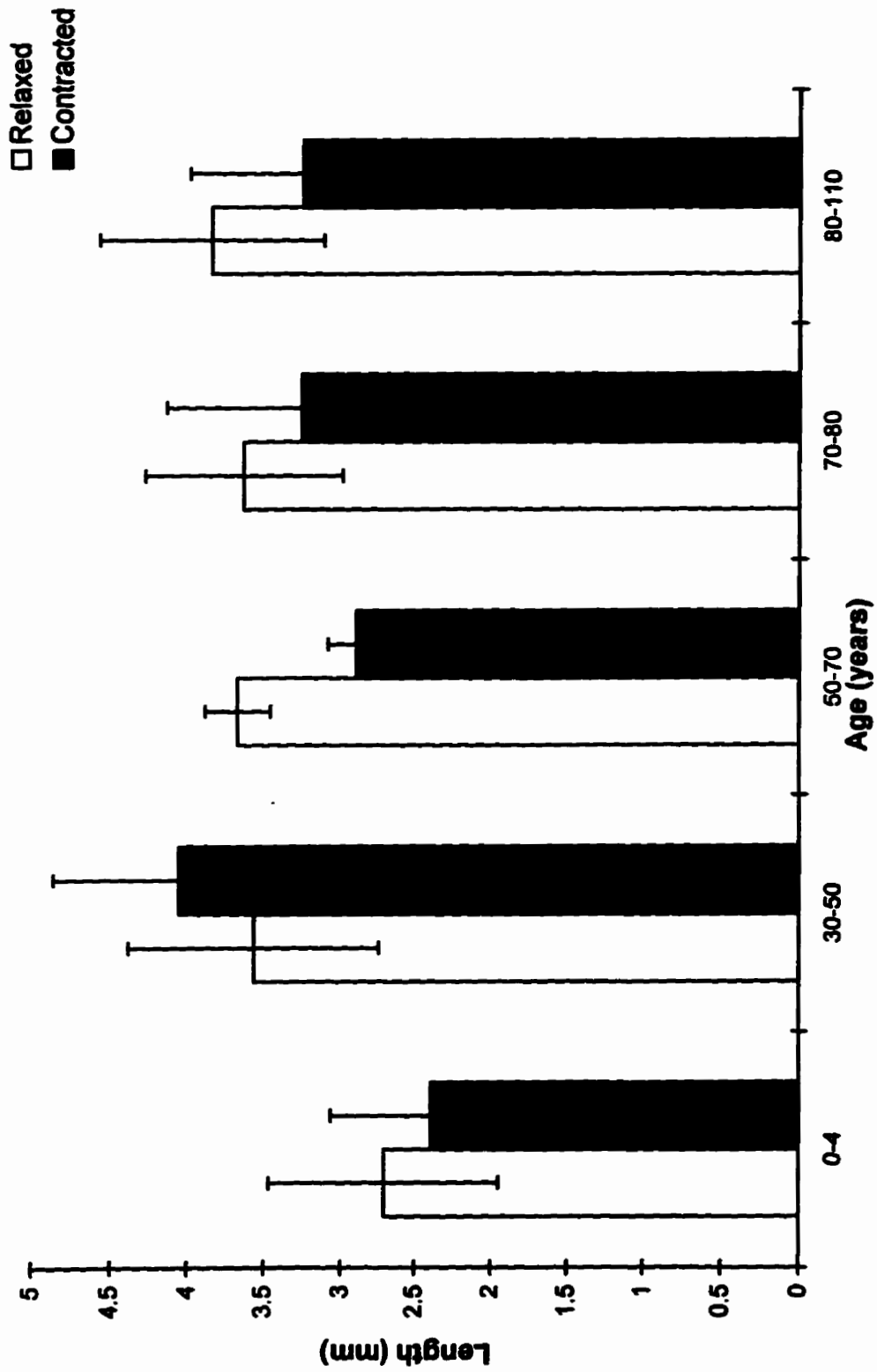
ciliary muscle function. The posterior length becomes significantly longer from 0-4 years to 30-50 years ($\bar{x}$ = 2.55 vs. $\bar{x}$ = 3.81 mm), then decreases significantly in the 50-70 year age group ($\bar{x}$ = 3.28 mm) and levels off with a slight upward trend (fig. 8a).

These measurements indicate that the ciliary muscle shows the largest change in muscle length with contraction (both total length, Δ = 21% and width to posterior length, Δ = 21%). The ciliary muscle appears to shorten in length and narrow in width with contraction in all age groups, except eyes from the 30-50 year old donors, where the ciliary muscle lengthens and widens with contraction. The opposite effect found in this age group is not the result of one donor, but at least two in each case (Table I). The eyes from 34 year old donor were consistently opposite in all measurements and this factor may contribute to the opposite results in this age group.

The ciliary muscle changes shape with age, with the muscle becoming shorter and wider after the age of 50 (fig. 11). Although the change in muscle length with age is not dramatic, the anterior length of the muscle is decreasing, indicating a forward shift of the ciliary processes in the eye. All of these age-related changes would result in the release of zonular tension on the lens as the ciliary muscle progressively brings the ciliary body forward and inward.

Proportion of muscle fibre groups

The relative proportions of circular, radial, and longitudinal fibres were measured to determine if the orientation of fibres changes with contraction, with age, and if the changes with contraction are dependent on age. In all three fibre types there is no significant change in the proportion of fibres with contraction and no relationship

Figure 11: Tracings of the ciliary muscle from a 42 year old (A and B) and an 85 year

old (C and D) in the relaxed (A and C) and contracted (B and D) states. Note the

change in length with contraction at both ages. With age the ciliary muscle is shorter

and the internal portion moves forward.

A

B

C
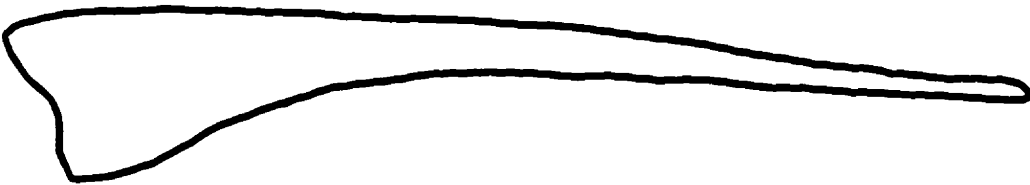
D

0.34 mm

between a change in the fibre orientation and age. There are, however, changes in the

percentage of radial and longitudinal muscle fibres with age, with the proportion of

circular fibres remaining constant. There is a significant increase in the percentage of

radial fibres with age. The proportion of radial fibres increases by 10.6% while the

longitudinal fibres decrease significantly by 12.5%, indicating that the muscle fibres

change orientation with age (fig. 12).

Proportion of muscle vs. connective tissue

The proportion of muscle versus connective tissue does not change with

contraction and there is no change in this relationship with age. However, there is a

significant decrease in the amount of muscle tissue in the ciliary muscle with age. The

proportion of muscle decreases from birth to age 107 from 75.9 to 52.0 % ($\Delta = 23.9\%$)

(fig. 13).

Figure 12: The proportion of muscle fibre groups
in the ciliary muscle vs age
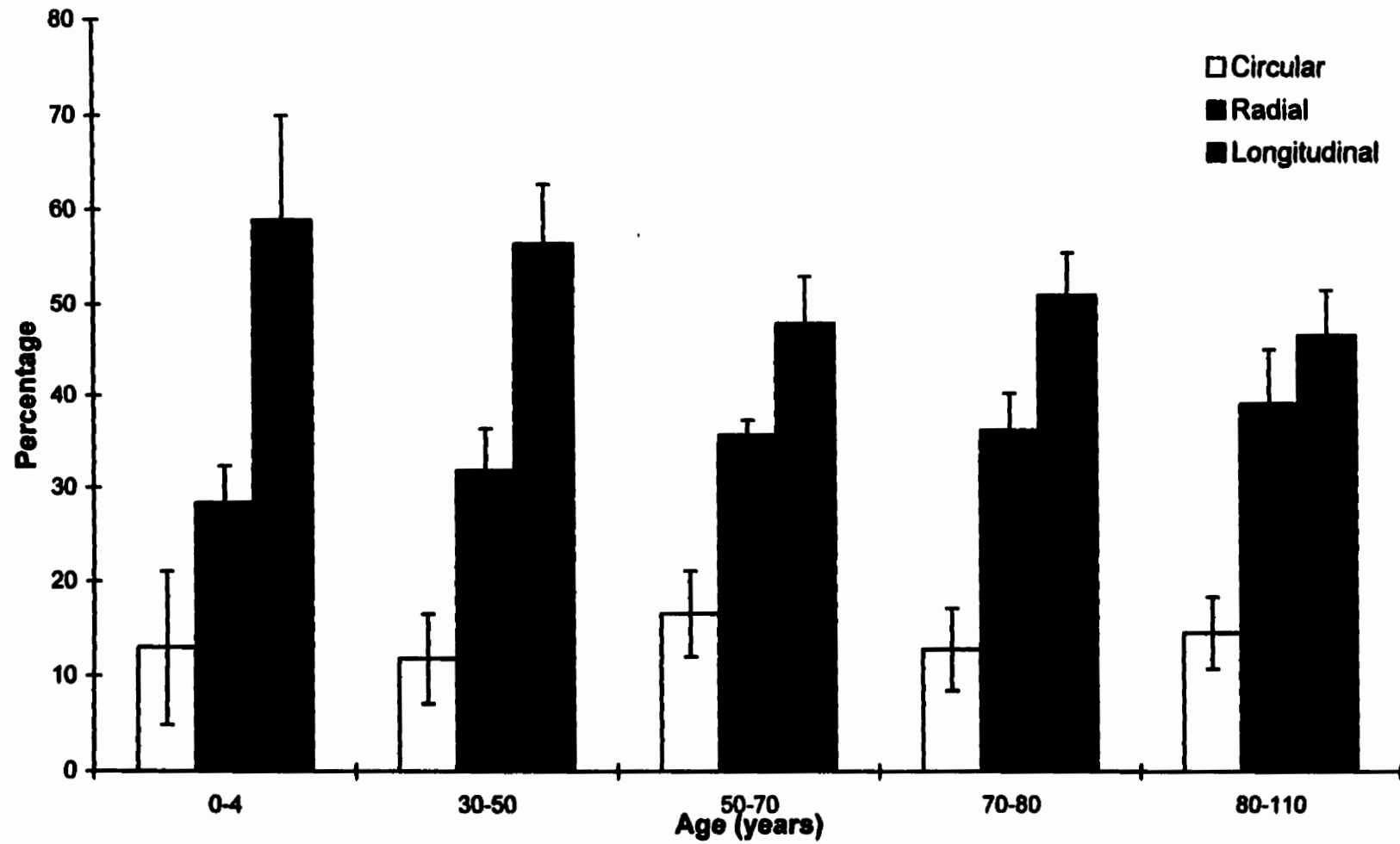
Figure 13: Proportion of muscle vs connective tissue in the ciliary muscle across age
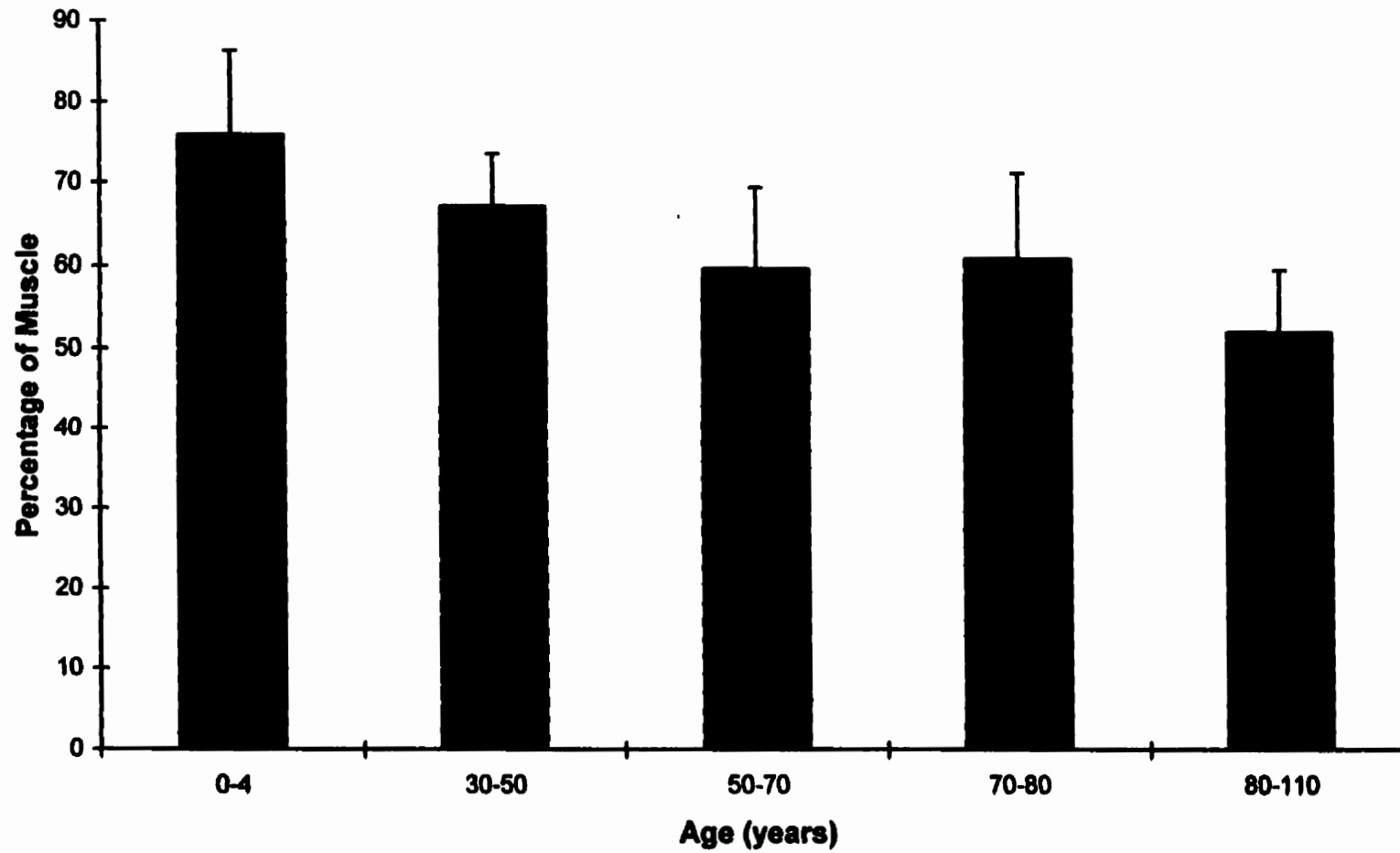
Asymmetry of the Ciliary Muscle

The ciliary muscle was analyzed for nasal and temporal asymmetry in eyes from 34 and 65 year old donors. Changes in the different regions were examined with contraction and changes in the regions with age, to determine if the ciliary muscle reacts uniformly around the eye.

Ciliary muscle dimensions, relaxed and contracted

A three-way repeated ANOVA reveals that the ciliary muscle is significantly shorter nasally than temporally ($\bar{x}$ = 3.98 vs. $\bar{x}$ = 4.59 mm). However, this difference is dependent upon the state of contraction and the age of the eye. In the nasal region of the ciliary muscle, the muscle shortens significantly with pilocarpine treatment (atropine-treated eye: $\bar{x}$ = 3.46 mm, pilocarpine-treated eye: $\bar{x}$ = 4.51 mm, Δ= 23%) (Table II), but in the temporal region the difference between the relaxed and contracted state is not significant (Δ= 4%). While both the nasal and temporal regions become shorter with age, the eyes from the 65 year old donor show a greater asymmetry between the regions (34 year old donor: nasal $\bar{x}$ = 4.88 mm, temporal $\bar{x}$ = 5.17 mm; 65 year old donor, nasal $\bar{x}$ = 3.09, temporal $\bar{x}$ = 4.00 mm) (Table II). Overall, differences between ages and differences between treatments in these two pairs of eyes show the same trends as those observed in the above analysis using data from all ages.

The width of the ciliary muscle is narrower on the nasal side (nasal region: $\bar{x}$ = 0.55, temporal region: $\bar{x}$ = 0.57 mm). Differences between the regions with treatment are not significant, indicating that the ciliary muscle is contracting uniformly around the

Table II: Measurements of Nasal-Temporal Asymmetry in the Ciliary Muscle

Measurement	Nasal	Temporal	Region of greatest change with contraction	Age with greatest difference between regions
Length	3.98 mm	4.59 mm	nasal	65
Width	0.53 mm	0.58 mm	n.s.	34
Anterior Length	0.54 mm	0.58 mm	n.s.	n.s.
Posterior Length	3.44 mm	4.01 mm	nasal	65
Circular Fibres	17.9 %	17.9 %	n.s.	neither
Radial Fibres	34.8 %	30.8 %	temporal	n.s.
Longitudinal Fibres	47.3 %	51.3 %	equal	65
Percent Muscle	58.2 %	57.2 %	temporal	n.s.

n.s. = not significant

eye. With age, the muscle is more asymmetric in the eyes from the 34 year old donor than eyes from the 65 year old donor (34 year old, nasal region: $\bar{x}$ = 0.59, temporal region: $\bar{x}$ = 0.63 mm; 65 year old, nasal region: $\bar{x}$ = 0.51, temporal region: $\bar{x}$ = 0.50 mm) (Table II). There is a general shortening with age, which is in agreement with the data for all ages. However, the muscle shows widening with contraction, not narrowing as in the other data. This may be due to orientation being controlled, although if this is true, one would expect there to be a significant difference in the two regions with contraction, and there is not.

The anterior length of the ciliary muscle is symmetrical around the eye and shows uniform contraction with treatment. The muscle also shows no significant differences in the two regions with age.

The posterior length has the same asymmetry as the total length of the muscle, the nasal region being shorter than the temporal one ($\bar{x}$ = 3.44 vs. $\bar{x}$ = 4.01 mm, respectively). The posterior length shows a greater shortening with contraction in the nasal region (relaxed: $\bar{x}$ = 2.88 vs. contracted: $\bar{x}$ = 4.01 mm) than in the temporal region (relaxed: $\bar{x}$ = 3.90 vs. contracted: $\bar{x}$ = 4.11 mm)(Table II). The muscle becomes shorter with age in both regions, although the eyes from the 65 year old donor show greater asymmetry (nasal: $\bar{x}$ = 2.74; temporal: $\bar{x}$ = 3.56) than the eyes from the 34 year old donor (nasal: $\bar{x}$ = 4.15; temporal: $\bar{x}$ = 4.46) (Table II). Analysis of the general change of posterior length shows the length increases with contraction. This is opposite to the results obtained with the other data, but can be explained by the large amount of lengthening with contraction in the eyes from the 34 year old donor (relaxed: $\bar{x}$ = 4.13

vs. contracted: $\bar{x}$ = 5.56 mm) (Table I). The eyes from the 34 year old donor may not be representative of the changes with contraction in this age group.

In general, ciliary muscle total length, width and posterior length are shorter in the nasal region (Table II). The nasal region shows greater changes with contraction in overall length and in posterior length than the temporal region. The eyes from the 65 year old donor show larger asymmetry between the nasal and temporal region in overall length and posterior length measurements, while the eyes from the 34 year old donor are more asymmetric in width of the muscle.

Proportion of muscle fibre groups

The proportion of circular fibres in the ciliary muscle is not different in the nasal and temporal region of the eye, nor is the proportion of circular fibres in these regions affected by contraction of the muscle. However, the proportion of circular fibres in these regions is dependent on the age of the eye. In the eyes from the 34 year old donor, the percent of circular fibres is greater temporally than nasally ($\bar{x}$ = 14.8 vs. $\bar{x}$ = 12.3 %, respectively), but in the eyes from the 65 year old donor, there are more circular fibres in the nasal region (nasal: $\bar{x}$ = 23.4 vs. temporal: $\bar{x}$ = 20.9 %). In the overall analysis of the effects of contraction, the percentage of circular fibres decreases with pilocarpine-treatment (atropine-treated eye: $\bar{x}$ = 20.5 vs. pilocarpine-treated eye: $\bar{x}$ = 15.2%). Overall, with age there is a greater percentage of circular fibres in the ciliary muscle from the 65 year old donor than in the muscle from the 34 year old donor($\bar{x}$ = 22.2% vs. $\bar{x}$ = 13.6%, respectively). These overall changes were not significant in the analysis

with the randomized wedges, possibly indicating that wedge position is important when comparing these measurements.

There is a significantly greater proportion of radial fibres in the nasal region of the eye (nasal: $\bar{x} = 34.8$ vs. temporal: $\bar{x} = 30.8\%$)(Table II). This difference is consistent between the two ages. A comparison of the atropine- and pilocarpine-treated eyes shows that there is a greater change in the proportion of radial fibres temporally (relaxed: $\bar{x} = 26.8$ vs. contracted: $\bar{x} = 34.8\%$) than nasally (relaxed: $\bar{x} = 33.8$ vs. contracted: $\bar{x} = 35.8\%$)(Table II). The overall change in the percentage of radial fibres with age is similar to the analysis of all ages. However, this asymmetric study reveals that the pilocarpine-treated eye contains a greater proportion of radial fibres than the atropine-treated eye ($\bar{x} = 35.3$ vs. $\bar{x} = 30.3\%$, respectively). The data for all ages showed no significant differences with contraction. This may have been a result of the fact that wedge position was not analyzed.

The proportion of longitudinal fibres is significantly greater in the temporal region of the eye (temporal: $\bar{x} = 51.3$ vs. nasal: $\bar{x} = 47.3\%$)(Table II). However, this difference is dependent on the state of contraction and the age of the eye. With contraction, the longitudinal fibres are more abundant in the nasal region and become less abundant in the temporal region. In other words, in the relaxed state, the percentage of longitudinal fibres is different between the nasal and temporal regions ($\bar{x} = 45.1$ vs. $\bar{x} = 53.2$ %, respectively), but in the contracted state there is no difference between the two regions (nasal: $\bar{x} = 49.5$ vs. temporal: $\bar{x} = 49.3\%$). With age the percentage of longitudinal fibres is more asymmetric in the eyes from the 65 year old donor (nasal: $\bar{x} = 36.6$ vs. temporal: $\bar{x} = 44.5\%$) than in the eyes from the 34 year old donor (nasal:

$\bar{x} = 58.0$ vs. temporal: $\bar{x} = 58.0\%$)(Table II). The summed analysis of age and treatment effects shows the same trend as seen in the data from all eyes. There is a decrease in the proportion of longitudinal fibres with age and no difference in the proportion of these fibres with contraction.

In summary, the differences in the proportion of fibre groups between wedges are not consistent. The proportion of radial fibres is greater in the nasal region, while the proportion of longitudinal fibres is greater in the temporal region (Table II). With contraction, the temporal region gains a larger proportion of radial fibres, but there is no change in circular fibres and the proportion of longitudinal fibres becomes more symmetric with contraction. The changes with age are also not consistent among the muscle fibre groups. The proportion of circular fibres is greater in the temporal region of the eyes from the 34 year old donor, but less abundant in this region in the eyes from the 65 year old donor. The radial fibres show similar changes with age, and the longitudinal fibres are more asymmetric in distribution in the eyes from the 65 year old donor.

Proportion of muscle vs. connective tissue

The proportion of muscle versus connective tissue is similar in the nasal and temporal regions of the ciliary muscle and is dependent on the state of contraction. After contraction, there is quantitatively more muscle tissue as measured by total surface area in the temporal region of the eye (nasal: $\bar{x} = 56.6$ vs. temporal: $\bar{x} = 52.4\%$)(Table II). This change is even more dramatic since the temporal region contains less muscle than the temporal region in the relaxed state (nasal: $\bar{x} = 59.9$ vs. temporal: $\bar{x} = 61.9\%$).

There is no significant change in the amount of muscle vs. connective tissue in the regions as a function of age. Overall, the proportion of muscle decreases with age, as reported above from the data of all ages. In the analysis of all ages there is no change in the percentage of muscle with contraction; however in this analysis, the relative proportion of muscle versus connective tissue decreases with contraction ($\bar{x} = 54.5$ vs. $\bar{x} = 60.9\%$). This finding indicates that wedge position may be important in showing significant differences in the quantity of muscle, as compared to connective tissue, when relaxed and contracted states are studied.

Discussion

General Ciliary Muscle Anatomy

There are two general theories on the arrangement of the ciliary muscle fibres. The first is that of Rohen (1964), who proposed that the ciliary muscle forms a reticulum where all the fibre types are interconnected. Thus, the longitudinal fibres turn and become radial which in turn become circular. In this theory, the proportion of fibre types changes with contraction. More circular fibres would form as the muscle contracted and pulled the ciliary body inward towards the lens. The second theory, that of Calasans (1953) suggests that the ciliary muscle fibres all form V-shaped bundles. The muscle fibres types are separate, but on contraction the radial fibres could possibly turn to a more circumferential position around the lens, appearing as circular fibres.

The current study, while identifying the three fibre groups, does not find evidence for a continuum of fibres in the ciliary body. As noted, the circular and radial fibres are particularly difficult to distinguish, especially in the younger eyes where the fibres do not form discrete bundles (fig. 3). Ciliary muscles examined in cross-section showed a similar pattern as in longitudinal section, with oblique fibres being very abundant, as determined by nuclei shape. The location and consistency of these transition zones indicate that the fibres groups are connected. However, instead of an interweaving of fibres, the fibres appear to be branching, perhaps in V-shaped bundles as suggested by Calasans (1953).

However, the connection between fibres types does not provide a point of fluidity between the fibre groups. When the ciliary muscle contracts, no change in the

proportion of muscle fibre groups is seen. Other studies have reported an increase in

circular fibres and a decrease in longitudinal fibres in humans (Rohen, 1964), primates

(van Alphen, 1963; Lütjen, 1966), and raccoons (Rohen *et al.*, 1989). The study of

human ciliary muscle compared eyes treated with pilocarpine and atropine. However,

neither the concentrations of drugs used, nor the ages or the number of eyes was given

(Rohen, 1964). In one primate study, 115 pairs of eyes were treated with a variety of

drugs (Lütjen, 1966). While this is an impressive number, the basis for classifying the

different muscles was not made clear. It appears that the extent of muscle contraction

was classified according to the proportion of the muscle fibre types seen, thus using the

expected outcome to categorize the muscles. All of the above studies which found

changes in the area of the fibres types did not describe the criteria for distinguishing the

three muscle fibre groups. The study by van Alphen (1963) demonstrates this point. In

two micrographs a line is drawn to approximate the area of circular fibres in the primate

ciliary muscle. However, the line does not appear to be drawn based upon fibre or

nuclei shape (the criteria used for the current study) but instead seems to assume that all

fibres in the internal bulge of the muscle are circular. The orientation of smooth muscle

fibres is hard to determine, unlike striated muscle fibres. Thus in order to determine the

direction of the muscle fibre, the plane of sectioning has to be determined. The easiest

way to do that is to classify the muscle fibre nuclei and fibre cells by shape, since it is

known that the nuclei in the human ciliary muscle cells is elongated and runs parallel to

the long-axis of the cell (Ishikawa, 1962).

The values for the different fibre types in this study roughly corresponds to the

values given for primates in the relaxed state (Lütjen, 1966). However, it seems

unlikely that the muscle did not contract since changes in ciliary muscle dimensions are seen. In a study of the aging of the primate ciliary muscle treated with 10% pilocarpine and 1% atropine, no circular fibres were present in the atropine-treated eyes, while the pilocarpine-treated eyes were described as having "well-developed" circular fibres (Lütjen-Drecoll, et al., 1988b). The concentration of pilocarpine and atropine used in this study (20 and 5%, respectively) are greater than used in the earlier described study.

Therefore, the muscle fibres of the human ciliary muscle are arranged in three fibre orientations and are connected by branching, possibly in a V-shape. The fibre types remain in the same plane with contraction. At birth the ciliary muscle contains all three fibre types but they are not grouped as in adults (fig. 3 vs. 4 and 6). The radial and circular fibres are intermixed and are described as not being fully developed (Hogan *et al.*, 1971). The circular fibres seen amidst the radial fibres, may be the precursors to the radial fibres.

The ciliary muscle is shorter in the nasal region in all the dimensions measured: total length, width, anterior length, and posterior length. Nasal-temporal asymmetry in the ciliary muscle and ciliary body is well documented in birds (Walls, 1942) and other mammals (pig: Rohen, 1964; horse: Henderson, 1926; raccoon: Rohen *et al.*, 1989). This study found, in addition, asymmetry in the proportion of muscle fibres groups: the proportion of radial fibres is greater in the nasal region while the proportion of longitudinal fibres is greater in the temporal region. The significance of nasal-temporal asymmetry may be related to bringing the lenses and therefore the visual axes of the two eyes closer. Accommodation is linked to convergence so that whenever the eyes focus

on a near object, the refractive power is increased and the line of sight is turned inward

towards the object (Moses, 1970). Thus, a more nasally placed visual axis results in less

movement of the eye in the orbit in order to focus while converging.

Ciliary Muscle and Accommodative Theories

While there are numerous theories on accommodation (see Atchison, 1995), the

only point common to all theories is the increase in refractive power of the lens with

ciliary muscle contraction. How the various accommodative structures act to produce

accommodation is still somewhat of a mystery. The lens has been theorized to thicken

(Helmholtz, 1909; Fincham, 1937) and thin (Tscherning, 1920; Schachar *et al.*, 1995),

while the zonular tension is decreased or increased, respectively, by ciliary muscle

contraction. An examination of ciliary muscle contraction may assist in determining

how the other accommodative structures are influenced by this force.

Data comparing the relaxed and contracted states, induced by atropine and

pilocarpine, respectively, show that the ciliary muscle becomes shorter and the width

decreases with contraction. The greatest change with treatment is in the total length and

posterior length of the ciliary muscle (21%) (Table I). This suggests that the

longitudinal fibres and to a minor extent the radial fibres are mainly acting in

accommodation. This contradicts the proposal that the longitudinal fibres mainly

function in outflow of aqueous fluid (Bill, 1967). The shortening of the ciliary muscle

would release tension to the zonules anchored to the pars plana.

The zonules are described as having four major tracts; 1) from the anterior lens capsule to the pars plana, 2) from the equatorial region of the lens to the ciliary processes, 3) from the posterior lens to the pars plana, and 4) from the posterior lens to the ciliary processes to the pars plana (Farnsworth and Burke, 1977). This main fibre system is anchored further to the ciliary epithelium of the pars plana by tension fibres (Rohen, 1979). Rohen hypothesized that the tension fibres hold the main fibres onto the curved surface of the pars plana. Thus the tension fibres are relaxed when the ciliary muscle is relaxed and the angle of the main fibres along the ciliary body is slight. However, with contraction, the internal surface of the ciliary body becomes more curved and the tension fibres would become taut to hold the main fibres against the ciliary epithelium. Rohen's theory also suggests that there is no movement of the posterior part of the pars plana during accommodation (Rohen, 1979). The data presented here does not support Rohen's theory since the posterior portion of the ciliary muscle is creating the main release of zonular tension. It seems more likely that the tension fibres, which course both anteriorly and posteriorly from the surface of the ciliary epithelium (fig. 5), simply assist the ciliary muscle in either relaxing or tightening the zonules. When the ciliary muscle relaxes, the posterior portion of the ciliary body would be pulled back and the tension fibres coursing posteriorly would assist in pulling the main fibres taut (fig. 14). When the ciliary muscle contracts, the ciliary body would shorten, pulling the tension fibres coursing anteriorly forward, which would assist in releasing zonular tension to allow for increased lens refractive power (fig. 14). The tension fibres may also assist in keeping the main fibres in a curve along the surface of the ciliary body,

however, the major advantage to the tension fibres would be the more even distribution of the force from the ciliary muscle along the zonules.

The release of the zonules by shortening of the ciliary muscle supports Helmholtz's theory of accommodation (Helmholtz, 1909). The data presented here also shows a narrowing of the ciliary muscle with contraction. Narrowing of the muscle width with pilocarpine-treatment has also been reported in primates (Lütjen-Drecoll et al., 1988b). Could this narrowing increase some zonular tension? Schachar et al. (1995) postulated that the ciliary muscle is pulling the equatorial fibres taut to increase the diameter of the lens while releasing tension of the zonules attached to the anterior and posterior surfaces of the lens. An examination of the ciliary muscle by the same group has concluded that the anterior portion of the ciliary muscle curls toward the sclera during contraction, which would keep the equatorial fibres taut. However, such a theory cannot be supported by the anatomy of the zonules, since the equatorial zonules are the least abundant and do not attach on the most anterior portion of the ciliary body.

While the decrease in width may at first appear to support the possibility of some increased zonular tension with contraction, the muscle width only changed by 4% and the muscle didn't consistently narrow in width at every age group. The ciliary muscle widened with contraction (16%) in the 30-50 year age groups (fig.9), which is the group with the largest accommodative amplitude (Duane, 1922; Banks, 1980). The lack of data between the ages of 4 and 34 makes it difficult to determine if the opposite reactions in the 30-50 year old age group are anomalous, or if they reflect muscle morphology and functionality of these eyes.

Figure 14: A schematic drawing of how the tension fibres may assist the ciliary muscle

(m) in changing zonular tension to the lens (L). In (A) the ciliary muscle lengthens

when relaxed which pulls the posteriorly coursing tension fibres (p) taut. The

posteriorly coursing tension fibres, in turn, pull the main zonular fibres (z) which flatten

the lens while the anteriorly coursing tension fibres (a) are slack. When the ciliary

muscle contracts (B), the ciliary muscle shortens in length, pulling the tension fibres

coursing anteriorly with it. This assists in pulling the main zonular fibers forward which

releases tension on the lens. The tension on the posteriorly coursing tension fibres is

also released.
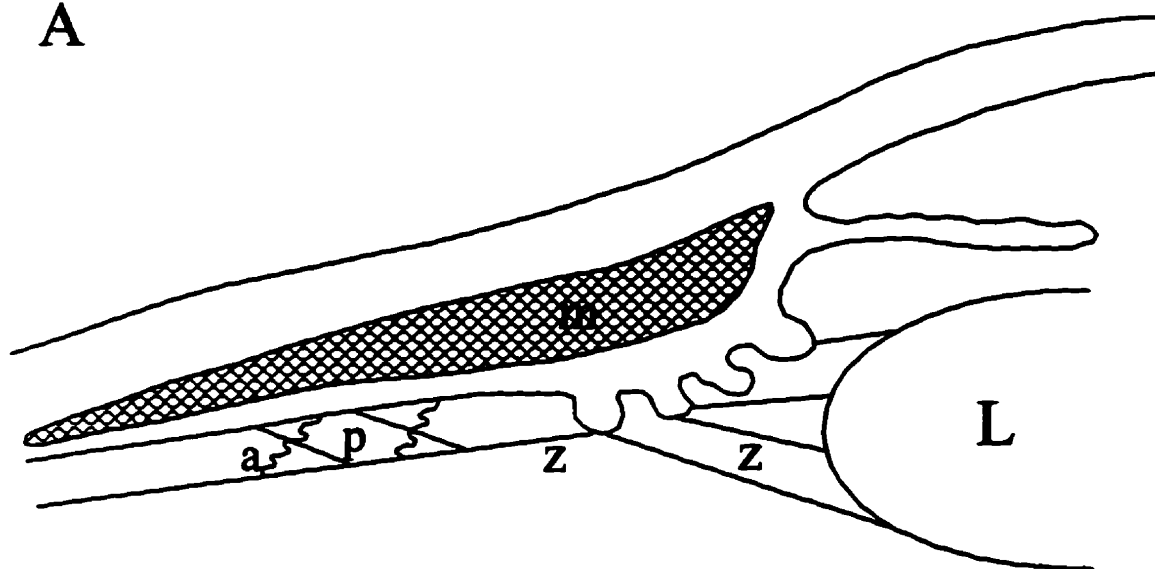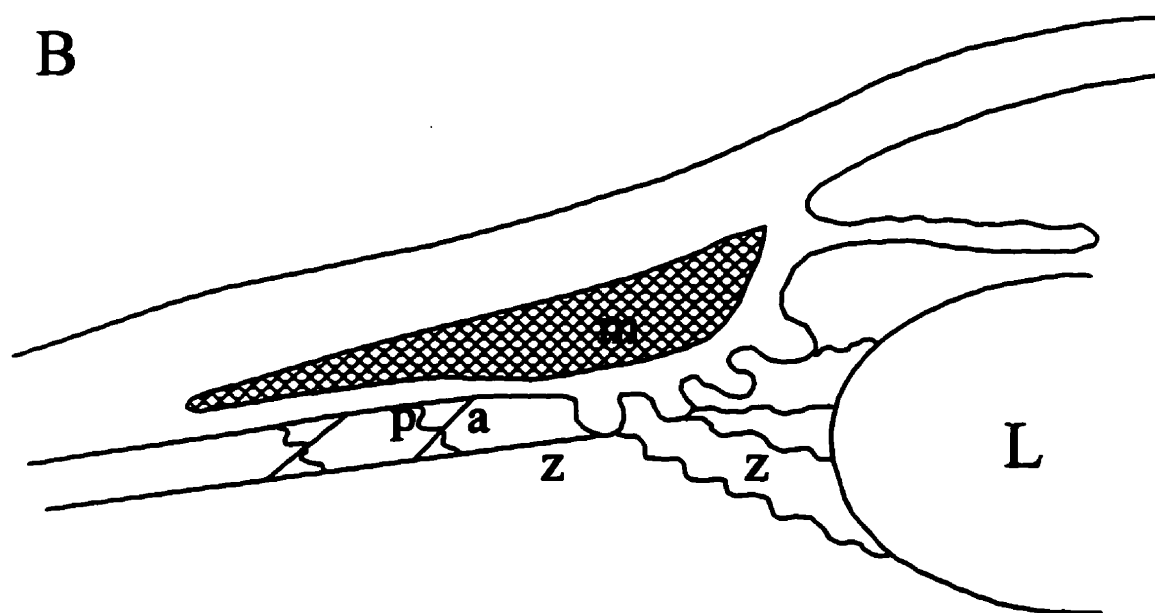
While the literature and the morphology of the muscle fibre groups suggest that the ciliary muscle is moving forward with contraction (Rohen, 1964; Lütjen-Drecoll *et al.*, 1988b; Bito and Miranda, 1989), this study found no differences between the anterior length of the ciliary muscle when comparing the relaxed and contracted states. This measurement notes the distance from the most anterior attachment point to the widest portion of the muscle and thus reflects the forward movement of the ciliary body with contraction. While the shape of the ciliary muscle is changing with contraction, the appearance of the forward movement of the ciliary body may be an illusion brought on by the shortening of ciliary muscle and the narrowing of the region where the radial fibres insert (fig. 4). Thus the main release of zonular tension is likely due to the shortening of the ciliary muscle, releasing tension on the zonules that attach in the posterior 2/3 of the muscle.

Changes in the Ciliary Muscle with Age

This is the first anatomical study of the human ciliary muscle to document no decline in muscle contractility as a function of age. Studies using indirect lens measurements (Fisher, 1977), impedence cyclography (Swegmark, 1969) and magnetic resonance imaging (Strenk and Semmlow, 1995) have also reported the unchanging mobility of the human ciliary muscle with age. However, studies of the primate ciliary muscles have shown an age-dependent decline in ciliary muscle mobility (Bito *et al.*, 1987a and b; Lütjen-Drecoll *et al.*, 1988b: and Neider *et al.*, 1990). These investigations have used video techniques, anatomical techniques, and slit-lamp

videography, respectively. Therefore, the difference in findings between humans and primates is not technique dependent, but appears to be a documented species difference.

However, the shape and composition of the ciliary muscle changes with age. The ciliary muscle becomes shorter in total length and posterior length after the age of 43. The width of the ciliary muscle consistently increases with age while the anterior length shortens after the age of 43 (fig. 11). These finding has been reported previously in humans (Tamm *et al.*, 1992) and also in primates (Lütjen-Drecoll *et al.*, 1988b). The widest part of the ciliary muscle is also moving anteriorly, indicating a forward movement of the ciliary muscle with age (fig. 11).

It is not clear from this study whether the forward movement and widening of the ciliary body is occurring independent of other age changes. The zonules move onto the anterior face of the lens with age (Farnsworth and Shyne, 1979) and the lens has been shown to become rounder and thicker (Brown, 1974). It is possible that the thickening of the lens and the forward movement of the zonules is pulling the ciliary body forward. However, it seems more likely that these changes occur simultaneously and together produce presbyopia. Certainly, the fact that the zonules would lose tension and allow the lens to become rounder, with anterior and inward movement of the ciliary body, could account for presbyopic changes. The eye would begin to lose accommodative function when the ciliary body can no longer produce adequate changes in zonular tension. The threshold for zonular tension to adequately change the shape of the lens is not known. While the changes described above may help explain how the lens becomes rounder with age due to the release of zonular tension, it does not explain why the "accommodated" lens becomes focused for more distance objects, a

phenomenon known as the lens paradox (Brown, 1974; Bito and Miranda, 1989). Thus, presbyopia is likely a multifactorial condition brought about by many simultaneously occurring age changes in the accommodative apparatus.

Other factors that may contribute to the loss of accommodation with age are the asymmetries that develop with age in the nasal and temporal region of the ciliary muscle. Of the two pairs of eye investigated for asymmetries, the 65 year old showed greater differences between the nasal and temporal region in total length, posterior length, and the proportion of longitudinal fibres than the 34 year old (Table II). This may indicate that the eye is contracting more asymmetrically with age and thus not releasing the zonular tension equally around the eye. This interaction (treatment by wedge by age) was not significant for any of the parameters measured, possibly due to the small number of eyes analyzed.

With age, the proportion of circular fibres remained constant while the radial fibres became more abundant and the longitudinal fibers less abundant. A similar study of the human ciliary muscle reported a decrease in the proportion of longitudinal and radial fibres and an increase in the proportion of circular fibres (Tamm et al., 1992b). Since the criteria for identifying the different muscle fibres types was not indicated, the difference between the findings cannot be explained.

In the previous study, an increase in the amount of connective tissue in the ciliary muscle was also reported, particularly in the region of radial fibres (Tamm et al., 1992b). The current study is the first to quantify the decrease in the proportion of muscle versus connective tissue from age 0 to 107 years of 24% with connective tissue more abundant in the internal regions of the muscle, the radial and circular regions (fig.

3 vs. 4 and 6). This gives the muscle fibres the appearance of being arranged in bundles and the differentiation between the fibre types is much easier in older eyes. In primates, connective tissue has been reported to increase mainly anteriorly, between the longitudinal and radial fibre regions of the ciliary body (Lütjen-Drecoll *et al.*, 1988b). The increase in connective tissue does not appear to have any effect on mobility of the human ciliary muscle. The loss of ciliary muscle mobility in primates has been accounted for by the thickening of the connective tissue in the posterior attachments of the muscle (Tamm *et al.*, 1992a; Poyer *et al*, 1993). As a result, much attention in the literature has been directed to an intact versus severed posterior attachment and its effect on contractility. In aging monkeys, an intact posterior attachment prevents forward movement of the ciliary body (Tamm *et al*, 1991; Tamm *et al.*, 1992a). Eyes used in the present study were embedded whole in methacrylate and then divided to help prevent this artifact. Of the 30 eyes analyzed, only 9 appeared to have a severed posterior attachment. However, no differences are seen between eyes with or without an intact posterior attachment. Thus, the aging of the posterior attachment does not limit ciliary muscle movement in humans.

Is the Primate a Good Model for Human Presbyopia?

In 1982 Bito *et al.* proposed the rhesus monkey as an animal model for presbyopia due to the decline in accommodative amplitude found with age. However, evidence is accumulating to the effect that the human and primate ciliary muscles do not change in the same way with age. With age, the human ciliary muscle becomes shorter,

wider and moves forward while the primate ciliary muscle becomes longer and narrower (Tamm *et al.*, 1992a). There is an increase in connective tissue with age in the ciliary body in both species. However, in primate eyes there is a relatively small increase in connective tissue within the muscle, but a large increase in the internal, anterior region of the ciliary body (ground plate) (Lütjen-Drecoll *et al.*, 1988a). In humans, the greatest increase in connective tissue is within the muscle, with relatively small increases on the internal edge of the ciliary body. And probably most significant is the decline in mobility of the primate ciliary muscle that is not seen in the human ciliary muscle. These differences result in questions regarding the application of findings in primate presbyopic research to humans.

Conclusions

The main effect of contraction is the decrease in length brought about mainly by the longitudinal and radial muscle fibres, which pull the zonules forward, with the assistance of the posteriorly coursing tension fibres. This action would likely release zonular tension on the lens, allowing it to become thicker. When the ciliary muscle relaxes, the zonules are pulled taut by the lengthening of the muscle and the anteriorly coursing tension fibres. This study found no difference in the proportion of muscle fibre groups between the relaxed and contracted state using the shape of the muscle cell nuclei to determine fibre orientations. Thus, the fibre orientations do not appear to be changing with contraction and are proposed to consist of three orientations of fibres that are connected by branching, but do not form a continuum.

No decline in ciliary muscle contractility is seen as a function of age. However, the ciliary muscle shortens and moves forward with age. This change in shape may release tension on the zonules and thus may explain the thickening of the lens with presbyopia. Although the ciliary muscle does not lose its ability to contract with age, the tension of the zonules may decrease due to the forward movement of the ciliary muscle, to the point where the action of the ciliary muscle would not alter zonular tension any further. Thus, the ciliary muscle is likely contributing to the development of presbyopia.

General Discussion

The aim of this thesis is to compare the functional anatomy of the ciliary muscle in birds and humans, two groups which accommodate by changing the shape of the lens while employing different accommodative mechanisms. This section discusses the similarities between the groups in terms of nasal-temporal asymmetry, the structures involved in accommodation, and the development of presbyopia. The discussion then addresses why the bird and human ciliary bodies differ by examining the evolutionary history and the visual demands of each group.

Nasal-temporal Asymmetry

Nasal-temporal ocular asymmetry has been documented for a number of vertebrate eyes including: reptiles (Walls, 1942), birds (Walls, 1942; Meyer, 1977; Murphy et al., 1995), horses (Henderson, 1926), pigs, dogs (Rohen, 1964), raccoons (Rohen et al., 1989) and humans (Hogan et al., 1971). This thesis provides additional detail concerning the nasal-temporal asymmetry in birds and humans. The nasal side of the ciliary muscle is shorter while the temporal side is longer. Other asymmetric characteristics include the pronounced separation of the muscle fibre groups in kestrels in the temporal region of the eye (see Section I). There is also the asymmetry of muscle fibre types in humans: the radial fibres being more abundant nasally and the longitudinal fibres more abundant temporally. In birds the ciliary muscle contracts uniformly around the circumference of the eye; but in humans, the change in length of the ciliary muscle is

greater on the nasal side. This asymmetry may be designed to increase the size of the binocular field (Walls, 1942).

In carnivores the eyes are located in a more forward position (falcons, lions, bears) while in herbivores the eyes are more laterally placed to provide a periscopic view of their surroundings (Hughes, 1977). The ciliary body, being shorter on the nasal side, displaces both the lens and the visual axis nasally allowing for a greater binocular field as well. Herbivores may also benefit from the increase in binocular field size caused by nasal-temporal asymmetry by being able to see frontally while eating and caring for their young. It has been suggested that the logic for carnivores needing a greater binocular field for running after prey does not follow through to primates, which for the most part do not chase their food (Hughes, 1977). In primates, the nasal-temporal asymmetry may be related to the fact that accommodation is linked to convergence of the eyes (Moses, 1970). Thus, a slight inward rotation of the visual axis due to the nasal-temporal asymmetry of the ciliary body may be of benefit in hand-eye related tasks.

Methods of Accommodation

Section I and II have addressed the role of the ciliary muscle in accommodation in birds and humans. In both groups lenticular changes are brought about mainly by the decrease in the length of the ciliary muscle with contraction. In birds, the muscle fibres shorten to pull the baseplate of the ciliary body forward, forcing the ciliary processes against the lens. In humans, shortening of the ciliary muscle releases tension on the

zonules and allows the lens to take on a more rounded state. While this study has concentrated on the role of the ciliary muscle in accommodation, other structures also may play a role in both groups.

There is evidence that the iris is involved in accommodation. In birds, the iris contains a sphincter (or circumferential), dilator and oblique muscle (Glasser *et al.*, 1995). In diving ducks, it has been proposed that the large iris sphincter muscle helps squeeze the lens in order to compensate for the neutralization of corneal refractive power underwater (Levy and Sivak, 1980; Sivak and Vrablic, 1982). In chickens, the peripheral iris muscle fibres assist in squeezing the anterior, equatorial region of the lens (Glasser *et al.*, 1995). Section I of this thesis has shown that the iris muscle fibres may function in accommodation in chickens and in hooded mergansers, while the anatomy of kestrel and pigeon iris musculature does not support the existence of such a mechanism.

The iris musculature of humans and primates consists of a smooth sphincter muscle and a dilator composed of myoepithelium (Freddo, 1996). An iris mechanism of accommodation has also been proposed in primates (Crawford *et al.*, 1990). Crawford *et al.* (1990) showed that accommodative amplitude decreases by 40% in iridectomized monkeys. The investigators hypothesize that the iris pulls the ciliary body more inward and anteriorly, to further release tension on the zonules. The iris is continuous with the anterior portion of the ciliary body and thus its ability to impose a force on the ciliary body is not unlikely. It should also be noted that the pupil constricts with accommodation. This has been explained as a mechanism to increase the depth of field (Moses, 1987). However, it may also serve to aid the action of the ciliary body in releasing zonular tension.

The cornea has been shown to have an accommodative role in some birds. The avian ciliary muscle fibres attach directly to the lamellae of the cornea. When the ciliary muscle contracts, the periphery of the cornea flattens, the central region steepens, and corneal refractive power increases (Glasser et al., 1994). Traditionally, in humans the cornea is not thought to contribute to accommodation. This is mainly based on the work of Young (1801) who used a fluid-filled contact lens to neutralize the power of the cornea and could detect no changes in the accommodative amplitude (Moses, 1970). Unlike the avian ciliary muscle which inserts directly into the cornea, the human ciliary muscle originates at the scleral spur, a rigid, circular ring located close to the canal of Schlemm at the periphery of the cornea (Hogan et al., 1971). Although the scleral spur is thought to be immovable, small changes in corneal curvature have been detected using pharmacological agents and normal accommodative stimuli (Roberts et al., 1993; Roberts and Ziavras, 1994 and Pardue, unpublished data). However, these corneal changes are not consistent as to direction (steepening or flattening) and they are only detected in the periphery of the cornea. Thus, central vision is not likely affected.

Many structures, not just the ciliary muscle, may be assisting in changing the refractive power of the eye. With the continuous arrangement of the structures in the enclosed environment of the eye, it seems unlikely that a single structure, like the ciliary muscle, would be able to function in isolation without affecting other structures.

Presbyopia

Section II of this thesis addresses the role of the human ciliary muscle in presbyopia, the age-related loss of accommodative amplitude. It has been shown that the development of presbyopia may not be the same in primates and humans as shown by the different ways the ciliary muscle ages. However, this doesn't diminish the fact that presbyopia occurs in primates and perhaps other vertebrates. In fact, an age-related loss of accommodative amplitude has also been documented in chickens (Sivak *et al.*, 1986) and pigeons (Glasser and Howland, 1995b). The cause of presbyopia is thought to be lenticular in both studies, although Glasser and Howland (1995b) did note a decrease in pupillary constriction in the eyes of older pigeons, suggesting a decrease in ability of the intraocular muscles to contract. It seems likely that a decline in the ability to accommodate also occurs in other animals. The loss of accommodation appears to be the only non-reproductive function completely lost before the end of the primate lifespan (Bito *et al.*, 1987). It would be interesting to know when age-related loss of accommodative amplitude occurs in other species in relation to their total lifespan. Presbyopia may not be seen in wild animals that depend on vision due to its disabling effects early in life.

While the preceding sections emphasize the similarities between the accommodative apparatus of humans and birds, there are distinct differences that reflect the groups' evolutionary histories and accommodative needs.

Evolutionary History

Duke-Elder (1961) describes the phylogenetic development of the ciliary muscle

as follows:

> The meridional (longitudinal) fibres are the descendant of the tensor
> chroidea of teleostean fishes and amphibians, and this becomes the ciliary
> muscle of reptiles (except snakes) and birds; in the latter it is unusually
> well developed and is divisible into two (Crampton's muscle, Brücke's
> muscle) and sometimes three parts (Müller's muscle). In the lower
> mammals the muscle is lacking or vestigial and is represented by a few
> meridional fibres. Only in the large-eyed placentals does it assume the
> prominent triangular shape characteristic of the human eye. In ungulates,
> which have little accommodation, it is represented merely by meridional
> fibres; in carnivores, in which accommodation is more active, the oblique
> fibres appear first; only in primates does the tripartite complexity of the
> muscle exist.

From this description, the ciliary muscle appears to have been derived directly from the

longitudinal fibres of the fish eye, without much change in the muscle, other than an

increasing complexity. This is somewhat misleading. For instance, the tensor

choroidea of fish, while perhaps being the precursor to the ciliary muscle in higher

vertebrates, is not involved in accommodation. This small muscle pulls the choroid and

retina around the vitreous body, holding it in place against the force of the backward

moving lens (Walls, 1942). Another muscle, the retractor lentis functions in

accommodation by moving the spherical, rigid lens towards the retina (Walls, 1942).

The retractor lentis is a smooth muscle composed of two fibre orientations that attach to

the ventral region of the eye and extend into the globe to insert onto the lens (Andison

and Sivak, 1994). From the retractor lentis muscle of the fish to the ciliary body

structure of higher vertebrates is a large jump. While it is not known how the vertebrate

eye evolved, the circular arrangement of the ciliary body would be needed in order to

apply equal force to change lens curvature.

Birds are thought to have evolved from Archosaurian reptiles while mammals are derived from Synapsid (mammal-like) reptiles (Olson, 1977). The early mammals are thought to have been nocturnal, relying mainly on scent and sound to feed upon insects and small invertebrates (Olson, 1977). The typical eye of a nocturnal mammal consists of a large lens, large pupil, and a rod-dominated retina. These characteristics maximize the collection of light and enhance the detection of movements from predators (Walls, 1942). Such species do not need to see objects sharply and do not need a well-developed accommodative apparatus. It has been hypothesized that the ciliary muscle of early mammals became rudimentary and then redeveloped as mammals became more dominant and fill more ecological niches (Walls, 1942).

Thus both birds and mammals may have originated from reptilian ancestors. The structure of the accommodative apparatus of these two groups reflects this common connection. The eyes of reptiles contain a wide range of accommodative structures, from the direct connection of the ciliary body to the lens in some lizards (Walls, 1942), to species such as the chameleon, where the ciliary body does not make direct contact with the lens due to the absence of ciliary processes (Pardue, unpublished observations). Instead, the connection is made via zonular fibres, as in humans. The ciliary muscle of the New Zealand reptile, *Sphenodon*, even more closely resembles the human ciliary muscle in that it contains two muscle fibre types: longitudinal and circular (Walls, 1942). Although the mammalian ciliary muscle is composed of smooth muscle, not striated, the muscle does have characteristics similar to certain evolutionary ancestors with striated ciliary muscles. However, it is not known if the ciliary muscle of early mammals completely disappeared during the evolutionary change from striate to smooth

muscle. Perhaps the zonules were retained to hold the lens in place while the ciliary

muscle disappeared. Then when mammals began to fill more ecological niches and

needed increased accommodative amplitudes, the ciliary muscle redeveloped as a

smooth muscle that released tension on the zonules to change the curvature of the lens.

Accommodative Needs

Birds and humans have a common evolutionary ancestor, yet the typical bird eye

is superior to that of other vertebrates in terms of accommodative range and resolution

ability (Duke-Elder, 1958; Shlaer, 1972; Fox et al., 1976; Meyer, 1977). The usual

range of accommodation in birds is about 20 dioptres (D) (Meyer, 1977). A red-tailed

hawk has been recorded to have as much as 28 D of accommodation (Pardue et al.,

1996), while diving ducks have been reported to have between 40-80D (Hess, 1912;

Goodge, 1960; Levy, 1979; Sivak et al., 1985). Birds use accommodation to hunt for

food and care for young. In raptors, accommodation may also by used for depth

perception when swooping down to capture prey (Lord, 1956). In humans, the

accommodative range in youths is approximately 14D at its maximum (Duane, 1922).

Humans do not need the large amounts of accommodative amplitude seen in birds, since

accommodation is mainly used for reading and hand related tasks at approximately arms

length. In birds the object of interest would be much closer (<10 inches), depending on

the species' size.

The large accommodative changes recorded in birds result from three factors. 1)

The force applied by the ciliary body contacts the lens directly, and thus can create

larger changes in lens curvature. 2) In some birds, like the hooded merganser, the iris may help squeeze the lens to create a larger refractive change (Levy and Sivak, 1980; Sivak and Vrablic, 1982). 3) In some birds the cornea also plays a role in accommodation . Since the cornea is the major refractive structure of the eye (Helmholtz, 1909), small changes in corneal curvature produce large changes in refractive power. In humans, as has been noted, the ciliary body indirectly attaches to the lens via the zonular fibres and thus the potential for lenticular changes is limited by the intrinsic roundness the lens can achieve by its own elasticity and the amount of tension the ciliary body can release. Probably the greatest accommodative difference between birds and humans lies in the area of corneal accommodation. In birds, corneal accommodation has been reported to contribute as much as 40-50% of the overall accommodative range (Troilo and Wallman, 1987: Schaeffel and Howland, 1987).

In addition, the striated nature of the avian ciliary muscle is responsible for a rapid reaction time for accommodation (0.03-0.08 seconds in ducks) (Sivak *et al.*, 1985). This may be compared to the human ciliary muscle which is composed of a multi-unit smooth muscle that takes approximately 0.5 seconds to contract (Moses, 1987) and approximately 2 seconds after onset of the stimulus to reach maximum (Alpern, 1969). Thus avian species have evolved a more powerful and efficient accommodative mechanism than exists in humans.

Conclusions

- The avian ciliary muscle can be divided into three muscle fibre groups based on fibre origin and insertion: 1) an anterior muscle fibre group that originates at the sclera under the scleral ossicles and inserts into the inner lamellae of the cornea, 2) a posterior muscle fibre group that originates on the sclera and inserts posteriorly into the baseplate of the ciliary body, and 3) the internal muscle fibre group which extends from the baseplate of the ciliary body to the inner lamellae of the cornea. The anterior fibre group pulls the periphery of the cornea back, altering corneal curvature and thus cornea refractive power. The posterior fibre group contributes to lenticular accommodation by pulling the baseplate of the ciliary body forward, squeezing the lens. The internal fibre group contributes to both corneal and lenticular accommodation by pulling the baseplate of the ciliary body forward and the periphery of the cornea backwards.

- Although the avian ciliary muscle is morphologically similar across species, there are species differences. Based on the anatomical investigation described in the first half of this thesis, chickens and pigeons accommodate by changing the shape of the cornea and lens. On the other hand, kestrels accommodate mainly by corneal curvature changes and hooded mergansers accommodate primarily by changing lens shape.

- The human ciliary muscle is divided into three fibre orientations: longitudinal, radial, and circular. While the fibres are connected by branching, evidence of a continuum was not found, as seen by the proportion of muscle fibres not changing with

contraction. The human ciliary muscle mainly shortens with contraction. This releases tension of the zonules, allowing the lens to take on a more rounded shape.

- The proportion of muscle versus connective tissue decreases in the human ciliary muscle with age. This does not affect the functional mobility of the ciliary muscle which remains constant throughout life. There is, however, an age-related shortening, widening, and forward movement of the ciliary muscle. These changes move the widest portion of the muscle forward and result in a decrease in zonular tension. It is proposed that these changes may be contributing to presbyopia by releasing zonular tension to the point that the action of the ciliary muscle no longer produces lenticular changes.

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