Fine structure of the human ciliary muscle

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The ciliary muscle in human eyes consists of bundles of muscle cells surrounded by flattened connective tissue cells. Each bundle generally contains unmyelinated nerve fibers accompanied by Schwann cells and a few connective tissue components. Some of the nerve fibers contain many vesicles characteristic of nerve endings. The nerve fibers are, in a few cases, in very close apposition to the muscle cell membrane, but in most instances they are separated by basement membrane material. Single muscle cells are, in general, cylindrical and entirely wrapped in a basement membrane except at small areas of contact with other muscle cells where it is absent. The central region of these cells contains a slender nucleus, centrosomes, Golgi apparatus, endoplasmic reticulum, and abundant mitochondria. Many myofilaments oriented parallel to the length of the cell axis are found in the cytoplasm. Elongated patches of dense material are observed between the myofilaments, particularly in contact with cell membranes. Numerous pinocytic vesicles occur along the muscle cell membrane. In the spaces between the bundles, collagen, very thin collagen-like (reticular) and elastic fibers, myelinated and unmyelinated nerve fibers, and capillaries are seen. The capillary walls are thicker than those of the ciliary processes, and lack pores.

In recent years, great numbers of investigators have paid attention to the fine structure of striated muscle, and many new findings and confirmations of classical ideas concerning its fine structure have been brought to our knowledge. However, the smooth muscle cell, having less striking features in comparison with striated muscle, has been the subject of only a few, some very brief, studies of the muscle in the uterus, urinary bladder, and artery.

The ciliary muscle in the eye plays an important role in accommodation, is very active and, therefore, probably differs in structure from smooth muscle found elsewhere. It also varies in structure in different species. The present study deals with electron microscopic observations of human ciliary muscle which extend those of Shiote, and reports further structural details, especially of the cytologic characteristics of the muscle cell, its morphologic construction, and the innervation of the ciliary muscle.

Materials and methods

Normal human ciliary muscles of 5 eyes obtained at operation were used in this study. Two
eyes were from persons 66 and 42 years old, were removed for small malignant melanomas of the posterior choroid, and the other eyes were 2, 4, and 7 years old; the first was removed because of a retinoblastoma and the other 2 for orbital rhabdomyosarcoma.

After enucleation, the eyeballs were immediately (within 2 to 3 minutes) cut into anterior and posterior parts near the ora serrata. The anterior half of the eye was immersed in cold fixative (2 per cent OsO₄ in veronal-acetate buffer, pH 7.4, with 0.045 Gm. sucrose per 1 ml. fixative), and the ciliary body was carefully stripped from the sclera. The ciliary body was dissected and fixed in fresh cold fixative for 2 hours.

After dehydration through an ethanol series, the materials were embedded in either Araldite or Epon 812 according to Luft’s procedure.

Sections for electron microscopy were made with a glass knife on a Porter-Blum microtome and were stained with uranyl acetate or a solution of lead.10 Counterstaining with a solution of lead, after very brief staining with uranyl acetate, was employed for a few sections. Electron micrographs were taken with Siemens Elmiskop 1 at original magnification of 2,500 to 12,000.

Observations

The smooth muscle cells of the human ciliary body are grouped in bundles. In each bundle, the muscle cells are closely packed and many peripheral nerve fibers are found between them. Each bundle is closely surrounded by a sheath of flattened fibroblasts (Figs. 1-6). Connective tissue, consisting mainly of collagen fibrils and fibroblasts, occupies the space between bundles. A small amount of connective tissue found inside the bundles consists chiefly of fine fibrils which may correspond to reticular fibers. In some instances, these fine fibrils are attached to the fibroblast.

Occasionally pigment cells are seen inside the bundles. Corresponding to the description based on the light microscopic observations some morphologic differences can be observed between the so-called meridional (the meridional fibers run anteroposteriorly and cross the equator of the globe at right angles) and the radial portion which is internal to the meridional fibers and fans out posteriorly to make a wide attachment to the connective tissue of the choroid.13

In the meridional part, clearly separated bundles of muscle cells run parallel to each other, and the intermediary connective tissue spaces are small (Figs. 1, 2, and 5). Therefore, in the meridional sections, as shown in Fig. 2, the muscle appears to consist of longitudinally disposed cylindrical bundles. In contrast in the radial part, the contour of the bundles is rather irregular and obscure and a dense connective tissue fills the spaces between the bundles (Fig. 3).

Moreover, the size and the shape of muscle cells differ in these two regions, i.e., the cells in meridional muscle have a relatively smooth outline and are about 3 μ in width at the widest part, usually the central area where the nucleus is located. Their length is too great to be estimated in these sections. In some cases one can follow a cell for over 20 μ even in a single section, whereas the cells in the radial part have an irregular outline which is approximately one and a half times as great in the nuclear region (Figs. 3 and 4).

The fine structure of smooth muscle cell in the human ciliary body is described below.

Cell membrane and basement membrane. Similar to the smooth muscle of the uterus, urinary bladder, and arteries, a single smooth muscle cell in the ciliary body is clearly bounded by a distinct cell membrane and by a basement membrane which has a thickness of about 250 A and which covers almost the entire cell closely, leaving only a few small areas uncovered. The interspace between the cell and basement membranes is constant, and approximately 150 A in width. In some regions, two basement membranes of contiguous cells fuse into a single membrane, or two cell membranes of contiguous cells share a single basement membrane (Fig. 5).

Frequently, the small areas where two adjacent cell membranes appear to be attached directly, without any interposing basement membrane, are observed as indicated by arrows in Fig. 6. The width of these areas is always less than 0.25 μ in
the plane of section. The cell membranes at such areas are thickened and show a somewhat desmosome-like structure. The basement membrane in these areas is continuous from one cell to the other. The contour of some muscle cell membranes is uneven, as shown in Figs. 2, 4, and 11. No myofilaments could be recognized in the convex portion of the cytoplasm of these cells.

A great number of vesicles, or caveolae, about 640 A in diameter are found along the cell membrane (Figs. 5 and 8). They may be a type of pinocytotic vesicle which suggests active transport in one or both directions.\textsuperscript{14}

**Nucleus and its vicinity.** The nuclei of smooth muscle cells are, in general, elongated in meridional and oval in the radial portions, respectively (Figs. 2 and 3). The nucleus is located in the central part with its axis parallel to the longitudinal axis of the cell. Occasionally indentations of the nuclear envelope can be seen (Fig. 8). The fine structure of the nucleus in ciliary muscle cells is very similar to that of smooth muscle cells described by other authors. The nuclear envelopes consist of double membranes separated by about 100 A in which pores occur only rarely. The interior of the nucleus is filled with a granular material scattered rather evenly and with an occasional slight aggregation of material observed along the inner nuclear membrane. Prominent nucleoli can be encountered (Fig. 8).

A small or indistinct Golgi complex has been reported in smooth muscle of several tissues.

The ciliary muscle cells, on the contrary, contain a well-developed Golgi apparatus which appears to be separated into several units, one in the vicinity of the elongated nucleus, generally at one side (Fig. 8). Single fragments are composed of 4 to 5 parallel Golgi membranes associated with numerous Golgi vesicles.

A pair of centrioles occurs frequently in the region enclosed by the nucleus and the Golgi apparatus (Fig. 8).

**Mitochondria and endoplasmic reticulum.** The mitochondria of ciliary muscle cells are rod shaped and occasionally bifurcated as shown in Fig. 8. Their diameter, approximately 0.2 \( \mu \), is relatively constant but their length varies considerably. The mitochondria are bounded by a double limiting membrane. The inner limiting membrane extends inward to form the closely packed cristae mitochondriales. The mitochondrial matrix is filled with a dense material.

The mitochondria tend to be concentrated on both sides of the elongated nucleus and in the central region of the cell, decreasing in number toward the end of the cell. The number of mitochondria is greater in the radial muscle cells than in those of the meridional cells. Fig. 7 shows the large number of mitochondria in a cell in the radial portion. It is remarkably different from that found in smooth muscle cells in other parts of the body.

The systematic arrangement of endoplasmic reticulum found in striated muscles\textsuperscript{15} is not seen in ciliary muscle cells. This material generally appears in tubular or vesicular shapes. Most of it belongs to the so-called smooth-surfaced endoplasmic reticulum. Fig. 9 shows part of a muscle cell found in the radial portion. In this illustration, considerable amounts of endoplasmic reticulum can be recognized. In most cases it consists of tubular or vesicular profiles which anastomose at some points. The content of reticulum or intracisternal material is amorphous and shows a higher electron density than that of the cytoplasmic matrix. The vacuolizations or dilatations of the reticular system found in some sections are probably artifacts produced during the preparation of the sections.

Most of the endoplasmic reticulum lies parallel to the mitochondria. In some places a connection between endoplasmic reticulum and cell membrane can be seen (Fig. 10). Over almost the entire cytoplasm, except where it is occupied by a mass of myofilaments, dense particles are observed.
They are 100 to 250 Å in diameter and heavily stained with the solution of lead. Most of these seem to be RNP-particles, the larger ones may be glycogen. Occasionally lipid droplets are seen in the cytoplasm of the muscle cells.

**Myofilaments.** In striated muscle, the myofilaments are regularly grouped into bundles, called myofibrils, and show clear cross-banding. Neither regular grouping nor cross-banding can be observed in the myofilaments of ciliary muscle cells. Myofilaments are, however, distributed throughout the entire cell and are arranged longitudinally (Figs. 9 and 10). In the transverse sections they appear as tiny dots and show relatively regular arrangement, i.e., hexagonal distribution at a distance of about 600 Å from each other (Fig. 5).

On examination of uranyl-stained sections at higher magnification, a single myofilament may be seen to be composed of two elements, a dense core about 80 Å in diameter surrounded by a faint filamentous material. These elements, however, are indistinguishable in longitudinal section. Between the myofilaments, particularly along the cell membrane, dense elongated bodies are found in every cell (Figs. 1 and 5). Both in transverse and longitudinal sections a fine filamentous structure can be observed around these dense bodies (Fig. 5).

**Nerve fibers and endings in ciliary muscle.** Many nerve fibers are very frequently observed in sections of ciliary muscle. The connective tissue spaces contain both myelinated and unmyelinated nerve fibers, which are always enfolded by cytoplasmic extensions of Schwann cells (Figs. 3, 5, and 15). Numerous unmyelinated nerve fibers with their Schwann cells penetrate between the muscle cells.

The diameter of nerve fibers in the bundles varies from 0.1 to 1.2 μ. Some nerve fibers are entirely wrapped by Schwann cells and contain fibrillar elements and a few mitochondria. The majority of the nerve fibers are rich in mitochondria and vesicles of 400 Å in diameter. These vesicles are similar to the synaptic vesicle found in synaptic endings elsewhere; therefore, it is reasonable that these also are synaptic endings. Furthermore, these nerve endings are not covered by Schwann cells, at least adjacent to the muscle. In very rare instances, tubular structures may be found in these synaptic endings (Fig. 10). In the ciliary muscle cells, three different types of neuromuscular junctions may be recognized with different frequencies. Fig. 12 shows the first type of junction. This type is the most frequently found so that it is seen very easily in every preparation. The main difference between this junction and the others is the intervention of the basement membrane between the membranes of the muscle and the nerve ending. The interspace between two synaptic membranes averages 800 Å in width, including the thickness of the basement membrane.

The second type may be found occasionally and is shown in Fig. 11. The main characteristics are the very close apposition of synaptic membranes and the small area of apposition. The intermembranous space is approximately 100 Å in width. In this type, a few muscle cells may be related to a single nerve ending.

The third type is found very rarely. In it, a large nerve ending, approximately 2 μ in diameter contacts the muscle cell over a relatively large area.

**Capillaries and connective tissue.** The space between the muscle bundles in the ciliary body is occupied by connective tissue, nerve tissue, and blood vessels. Generally capillaries are situated outside the bundles, but occasionally they come close to the muscle cells (Figs. 1 and 14). The capillary walls are thicker than those of the ciliary processes and usually lack pores. The connective tissue spaces are occupied mainly by the collagen fibrils, which show the characteristic periodicity of 640 Å. The flattened fibroblast, pigment cell, very thin collagen-like fibers, and the elastic fibers which often appear in older ciliary bodies are also seen.
Fig. 1. Slightly oblique, transverse section of a meridional portion of human ciliary muscle. The muscle cells (M) are grouped in flattened bundles which are surrounded by thin, flattened fibroblasts (F). Between the bundles, nerves (n) and a capillary (Cap) are seen. P, Pigment cell. Specimen age, 2 years. (×10,000.)
Fig. 2. Longitudinal section of a meridional portion of human ciliary muscle. Slender muscle cells (M) are packed closely to and parallel with each other. The connective tissue components (con) are sparse and pigment cells (P) are seen within the bundles. Specimen age, 7 years. (×7,200.)
Fig. 3. Cross section of the radial portion of ciliary muscle. Bundles cannot be clearly distinguished from each other. Many collagen fibrils (cf) are seen in the connective tissue space. M, Muscle cells; n, Nerve fibers; F, Fibroblasts. Specimen age, 42 years. (×9,300.)
Fig. 4. For legend see opposite page.
Fig. 5. Cross-section of meridional muscle cells. Muscle cells contain mitochondria (m), endoplasmic reticulum (ER) and myofilaments (myo) distributed throughout the cytoplasm. Dense bodies (arrow) are seen between the myofilaments and along the cell membrane. A single muscle cell is almost entirely covered by basement membrane (Bm). Fine collagen-like fibrils (f) are seen in the bundle and in the connective tissue space. n, Nerve fibers. Sch, Schwann cell. F, Fibroblasts. (x17,400.)

Fig. 4. Longitudinal section of the radial portion of the ciliary muscle. Muscle cells (M) show more irregular profiles and have a larger cell body than have the meridional fibers shown in Fig. 2. Nerve fibers (n) are accompanied by Schwann cells (Sch). F, Fibroblasts. Specimen age, 4 years. (x8,600.)
Fig. 6. Cross-section of meridional muscle cells. The small areas indicated by arrows show the direct attachment of adjacent cells with no basement membrane (Bm) between two cells. Basement membrane in the area covering is continuous from one to the next. F, Fibroblast. m, Mitochondria. n, Nerve fibers. (×28,000.)

Fig. 7. Slightly oblique, longitudinal section of radial muscle cells (M). Large numbers of mitochondria (m) are present in the cytoplasm, especially near the nucleus (N). cf, Collagen fibrils. F, Fibroblast. (×17,000.)

Fig. 8. General survey of a part of the nuclear region of a ciliary muscle cell. Nucleus (N) shows an indentation and has a prominent nucleolus (Nl). A well-developed Golgi apparatus (G) and a pair of centrioles (C) are seen at this region. Along the cell membrane, many pinocytotic vesicles (v) are observed. m, Mitochondria. Bm, Basement membrane. (×34,800.)

Fig. 9. Higher magnification of a part of a ciliary muscle cell from the radial portion, showing mitochondria (m), endoplasmic reticulum (ER), and myofilaments (myo). (Note that the mitochondria and endoplasmic reticulum are located near each other.) Numerous free RNP particles (p) are seen in the cytoplasm except between myofilaments. (×29,000.)
Fig. 7. For legend see opposite page.
Fig. 8. For legend see page 596.
Fig. 9. For legend see page 596.
Fig. 10. Longitudinal section of muscle cells (M) and nerve endings showing Type 1 neuromuscular junction. In the synaptic ending, tubular structures (ts) are seen in the nerve as well as synaptic vesicles (sv) and mitochondria (m). Endoplasmic reticulum in a muscle cell is shown crossing myofilaments (myo) and attaching to the muscle cell membrane (arrow). (x20,800.)
Fig. 11. Neuromuscular junction Type 2. The nerve ending containing synaptic vesicles (sv) and mitochondria (m) is directly attached to muscle cell (M) membranes in a small area. No basement membrane is seen between the nerve ending and the muscle cell membranes. Three junctions (arrows) are seen on a single nerve ending. Note folds in muscle cell membrane, left side of figure. (×20,800.)
Fig. 12. Neuromuscular junction Type 1. A nerve ending containing many synaptic vesicles (sv) and mitochondria (m) is in apposition to the muscle cell (M) membrane, with an intervening basement membrane (Bm). The nerve ending is not covered by a Schwann cell (Sch), at least on the side adjacent to the muscle. (×26,000.)
Fig. 13. A large nerve ending containing synaptic vesicles (sv) and mitochondria (m) is seen in a depression in the muscle cell (M) with a large attachment area without basement membrane (Bm). N, Nucleus. (×28,000.)

Fig. 14. Muscle cells (M) and blood capillary (Cap) containing erythrocytes (E). A capillary is located close to the muscle cell with no intervening fibroblast. Endothelial cells (En) of the capillary are thin but lack pores. Many pinocytotic vesicles (v) are seen along the cell membranes of muscle cells and the endothelial cell. (×15,600.)
Fig. 15. A part of the connective tissue in the ciliary body showing a capillary (Cap) and myelinated (mn) and unmyelinated nerve fibers (n). Small peripheral nerve fibers also are enfolded by Schwann cells (Sch). Collagen fibrils (cf) and a fibroblast (F) are seen in the connective tissue space. (×15,600.)

Fig. 16. A part of an arteriole found in the ciliary body. The smooth muscle cells (M) around the vessel contain only a small number of cytoplasmic organelles. Collagen fibrils (cf) and elastic fibers (ef) are seen around the vessel. En, Endothelial cell. (×18,200.)
Discussion

According to the descriptions of the uterus\(^1\)\(^-\)\(^3\) and arteries\(^4\)\(^-\)\(^6\), their smooth muscle cells are more or less enclosed by the intercellular collagen fibers. The cellular sheath found in ciliary muscle was not reported in the description of other smooth muscles. The fibroblast sheath in the ciliary muscle may correspond morphologically to the endomysial cell in the muscle spindle\(^17\) or to the Henle sheath in nerve tissue. Functionally, on the other hand, it might be possible to assume that the sheath acts as a diffusion barrier, as do the capsular sheath cells in muscle spindles or in epineurium.\(^18\)

According to the explanation by Shiose\(^8\) muscle spindle-like tissue was distinct from that of "ordinary" muscle bundle. However, every muscle bundle in ciliary muscle possesses a more or less muscle spindle-like structure as shown in the present observation. Nevertheless, no distinct capsular-sheet cells can be recognized in the
There is a morphologic basis for suggesting that the ciliary muscle is composed of physiologic units, i.e., the bundles of several smooth muscle cells. These aggregates, separated from the others by fibroblast cytoplasm, contain nonmyelinated nerves and nerve endings which may be related to more than one cell, but only those of one bundle.

The muscle cell in the ciliary body has a distinct cell membrane and it seems to be certain, based on this material, that individual cells are entirely separated from each other. At the area of most direct contact between adjacent cells in the ciliary muscle, the cell membranes are not separated by a basement membrane, and there is a cell membrane to membrane apposition. This structure is similar to that of the intercalated disc in cardiac muscle and therefore probably may be assumed to be the site of a firm attachment device and of a pathway of conduction from cell to cell.

The irregular contour of the muscle cell membrane shown in Figs. 4 and 11 may be interpreted as indicating contraction. Although there is no direct support of this, the absence of myofilaments from the folded portion strongly suggests this interpretation.

Electron microscopic studies of uterus and artery smooth muscle cells showed them to contain relatively small numbers of organelles, mainly near both ends of the elongated nucleus. The smooth muscle of the arteries in the human ciliary body and the sphincter muscle of the iris, which were obtained from the same eyes used in the present study, also have many characteristics similar to those of the uterus mentioned above. In contrast, the main characteristic of the fine structure of human ciliary muscle is the abundance of mitochondria and endoplasmic reticulum compared with ordinary smooth muscle. This characteristic is more conspicuous in the radial portion. Generally, the smooth muscle fibers of the ciliary body are reported to be aggregated in three groups according to the direction of their long axes, meridional, radial, and circular portions. In general, the muscle cells of the first two portions differ from each other in size, shape, and fine structures as well as in localization. For convenience’s sake, these two types of cells were described respectively in previous observations. However, such characteristics are not distinct in each part, but change rather gradually; therefore, in some parts of the ciliary muscle, especially in the middle portion of the meridional and radial portions, the muscle cells have intermediate characteristics in all respects. In the circular portion of the ciliary muscle, the muscle cells may not be distinguishable from those of the previous two portions in their structural characteristics except by their location. In other words, muscle cells in the circular portion have also “intermediate characteristics” in shape, size, and fine structure.

The relatively large number of cytoplasmic organelles, especially the large numbers of mitochondria, might suggest that ciliary muscle cells possess higher enzymatic and functional activity than those of other regions. The rich endoplasmic reticulum in ciliary smooth muscle is not arranged in a systematic pattern, as in striated muscle, but is distributed to form a framework in cells which possess grouping of the myofilaments. In such cases, it can be presumed that it has a function similar to that of sarcoplasmic reticulum.

A single type of myofilament varying from 30 to 80 Å in diameter has been described in smooth muscle cells of various tissues by several authors. In uranyl-stained preparations of ciliary muscle, myofilaments can be observed as a complex of dense and faint filaments. Further study of the myofilaments of smooth muscle is indicated.

A dense elongated body found by almost all observers in smooth muscle generally is also distinct in the ciliary muscle. Based
on the present study, it seems reasonable that this structure is an attachment device of the myofilaments, as suggested by Pease and Molinari.5

Boeke20 in 1932 described innervation of ciliary muscle as follows: "As far as can be made out, here every single muscle cell seems to have its own nerve termination." In this electron microscopic study, numerous nerve fibers are observed, most of which appear to be nerve endings. It is impossible to estimate the number of innervations in the ultrathin sections. The frequency of occurrence of nerve endings in single sections is very much higher in ciliary muscle than in smooth muscle elsewhere. Three types of neuromuscular junction could be recognized in the ciliary muscle in this study. The first is an indirect type of contact of synaptic membranes with an intervening basement membrane. The majority of nerve endings belonging to this type were also found in the smooth muscle of arterial walls,21 in uterus,4 and ciliary muscle.8 The second type is a more intimate contact of nerve and muscle without an intervening basement membrane. This type of contact is not too frequent and was not reported in smooth muscle previously. In the third, the nerve ending may be seen to lie in a depression in the muscle cell and to attach closely to the muscle membrane over a relatively large area. This type was reported in uterine muscle by Caesar and associates4 and in muscle coat of rat vas deferens by Richardson22 and occurs rarely in ciliary muscle. In such cases, most of the myoneural junctions belonged to the first type.

Most blood vessels in the ciliary body are capillaries. A few small arterioles and small venules are found. The endothelium of the capillaries in the ciliary body is thin, although thicker than that of the ciliary processes or of the subepithelial spaces, and has no pores. The structural difference between capillaries in the ciliary processes and the ciliary muscle might represent a functional difference.

An attempt has been made to summarize much of the information obtained from the study of these electron micrographs in a schematic drawing, Fig. 17.

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