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Insertion of the Extrinsic Eye Muscles of Vertebrates.

Aeleta Nichols Barber

Louisiana State University and Agricultural & Mechanical College

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INSERTION OF THE EXTRINSIC EYE MUSCLES OF VERTEBRATES

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Zoology

by

Aeleta Nichols Barber
M.S., Emory University, 1936
August, 1948
ACKNOWLEDGMENTS

I wish to thank Dr. Charles M. Goss for suggesting the problem and for his kindly advice and criticisms of my results. I also wish to express my sincere appreciation to Dr. William H. Gates and Dr. Russell L. Holman for their valuable help and encouragement in all my problems. My thanks are also acknowledged to Dr. William G. Nothacker for the photomicrographs used to illustrate this paper.
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ABSTRACT

Insertion of the Extrinsic eye Muscles of Vertebrates

A review of the historical background of the subject reveals two conflicting viewpoints. According to one, the muscle fiber is enclosed in a protoplasmic sheath, the sarcolemma, which forms a limiting membrane between it and the tendon. The other view denies the existence of this limiting membrane and considers the tendon fibrillae as extensions of the muscle fibrillae. A compromise view states that, although the sarcolemma is present at the tip of the muscle fiber, it is pierced by the myofibrillae which are continuous with the tendon fibrillae. Recent researches on the morphology of the attachment of skeletal muscle has established the argyrophilic nature of the tendon fibrillae by the use of a reticulum stain.

This paper endeavors to answer five questions. (1) Is the relation of muscle fibrillae to tendon fibrillae the same in the extrinsic eye muscles of all classes of vertebrates? (2) Does the relationship of the myofibrillae to tendon fibrillae in these muscles differ from that described for other muscles? (3) Are the myofibrillae continuous with the tendon fibrillae in the extrinsic eye muscles of vertebrates? (4) Can the sarcolemma be demonstrated as a membrane distinct
from the argyrophilic network surrounding each fiber? (5)
Are other tissues such as elastic tissue, concerned in the
muscle-tendon attachment of eye muscle?

The lateral rectus muscle of adult animals was used
throughout the investigation. The animals studied were fishes,
amphibia, reptiles, birds and mammals. Serial sections were
stained by a reticulum-connective tissue technique and the
Verhoeff method for elastic tissue. In this presentation
tendon is defined as the argyrophilic fibrillae which
intervene between the muscle fiber and the collagenous
elements of the sclera.

The argyrophilic nature of the tendon fibrillae was
demonstrated by comparing sections of the same muscle stained
by various combinations of the reticulum-connective tissue
method. By the combined stain the muscle fibers are red, the
collagenous elements green and the reticular fibrils black.

These sections were compared with others stained by the silver
component alone in which the reticulum network is revealed
as black threads against a clear background and with those
stained by the connective tissue component alone in which
no hint of the argyrophilic nature of the tendon fibrillae
or reticulum can be detected.

Comparison of these sections indicates that the
relation of myofibrillae to tendon fibrillae in the extrinsic
eye muscles is essentially the same in several classes of
vertebrates and is similar to that described for skeletal
muscles in other parts of the body. Also, the argyrophilic nature of the tendon fibrillae was demonstrated and, in no instance was the silver stained fibrillae found to be inside the sarcous portion of the muscle fiber.

The presence of a sarcolemma, or protoplasmic membrane, distinct from the argyrophilic membrane could not be demonstrated in any of the preparations studied. The appearance of a clear, transparent membrane was observed in sections stained by the connective tissue component alone but adjacent serial sections stained by silver nitrate reveal this structure to be argyrophilic in nature. In no instance, even in those cases where the outer covering or sarcolemma was detached from the muscle fiber during preparation of the tissue, was there any structure or membrane which did not stain black with the silver stain.

The elastic tissue content of the extra-ocular muscles was shown to be uniformly negative by the Verhoeff method for elastic tissue. However, the samples studied were considered inadequate and further investigation on this aspect of the problem is planned.

The author concludes (1) that the morphology of the insertions of the extrinsic eye muscles of vertebrates is essentially the same and similar to other muscles of the body, (2) that the muscle fiber is entirely enclosed by an argyrophilic net and inserts into the collagenous tissue of
the sclera through the intervention of these argentofibrillae, and (3) that the elastic tissue content of the extrinsic eye muscles of vertebrates appears to be less than that usually described in the literature.
The attachment of skeletal muscle has been a controversial subject since 1732. The early anatomists described the tendon as a continuation of the muscle fibers. Since that time improved methods of observation have permitted the differentiation of the sarcolemma and the sarcoplasm containing the delicate myofibrillae from the coarser fibrils which form the tendon. However, during this period two conflicting theories and one compromise view developed in regard to the intervention of the sarcolemma between the myofibrillae and the tendon fibrillae. According to the sarcolemma theory, the muscle fiber is enclosed in a protoplasmic sheath, the sarcolemma, which forms a limiting membrane between it and the tendon. On the other


hand, the continuity theory denies the existence of this limiting membrane and considers the tendon fibrillae as extensions of the muscle fibrillae. A compromise was offered by Schultz to the effect that a sieve-like plate exists through which the myofibrillae are continuous with the tendon fibrillae. Comprehensive reviews of both sides of this controversy are given by Carr for continuity, and by Häggquist and Long for the sarcolemma theory.

The purpose of this paper was to compare the relationship of the muscle fibrillae to tendon fibrillae in the same


6 Schultz, op. cit.
8 Carr, op. cit.
3 Häggquist, op. cit.
5 Long, op. cit.
muscle of a series of adult animals from as many classes of vertebrates as possible. The relationship of muscle to tendon has been investigated from the standpoint of morphology and histogenesis and by numerous techniques, such as tissue culture, microdissection, electric stimulation and innumerable histologic techniques. Recently Goss studied the morphology of the muscle fiber by the use of a combined reticulum-connective tissue stain and Long applied the same technique to a study of the histogenesis. Goss used different muscles from a single species, rhesus monkey, Baldwin, although he did not use the same methods, described the morphology of various muscles from several species of vertebrates, and Downey and Jordan investigated the condition in invertebrates. However, Baldwin compared the muscles of immature animals with others from adult animals.

6 Schultze, op. cit.
8 Carr, op. cit.
4 Goss, op. cit.
5 Long, op. cit.
2 Baldwin, op. cit.
I decided to use the extrinsic eye muscles of vertebrates for several reasons. In the first place, I am primarily interested in ophthalmology and only a few very incomplete descriptions of the histology of the extra-coular muscles are available in the literature. Also I felt that a study of the insertions of the same muscle in adult animals of a phylogenetic series would offer additional information on the relation of striate muscle fibrillae to tendon fibrillae. Since these muscles insert directly into the sclera it was easy to preserve the relationship of the muscle fibers to the site of their insertions without extensive dissection or decalcification of bone.

Material and Methods

The lateral rectus muscle of adult animals was used throughout this investigation. All the animals, except the fish, were etherized in order to secure the muscle in a relaxed condition. Urethane was used to anesthetize the fish. The eyes were dissected under a dissecting microscope and the specimens oriented in such a manner that the plane of the section would pass longitudinally through the tip of the muscle and its attachment to the sclera. The specimens were fixed in 10% neutral formalin (4% solution of formaldehyde) for 42 hours and embedded in hard paraffin (M.P. 59°C). Serial sections were cut at 5 micra and stained by various combinations of a reticulum-connective tissue stain and a
stain for elastic tissue. Groups of serial sections were stained by silver nitrate alone and show the argentofibrillae as black threads against a clear background. Other groups were stained by the connective tissue component alone which consists of Ponceau de Xylidine and acid fuchsin with light green. The muscle fibers are red and the collagenous fibers green in these preparations but the reticular elements do not stain at all. The combination of silver nitrate with the connective tissue stain is exacting but the results are very consistent. An excellent discussion of this method is given by Long.\(^5\) Serial sections were also stained by the Verhoeff method for elastic tissue (Mallory).\(^13\)

Observations

In the eyes of fishes, amphibians, reptiles and birds the sclera is characterized by a layer of cartilage which serves as a support for the choroid and retina. In fishes, amphibians and reptiles this usually takes the form of laterally situated plaques; in birds it is more usual to find a ring-formation in the posterior part of the eye with a perforation to allow the passage of the optic nerve. No such condition is found among the mammals with the exception

\(^5\) Long, op. cit.

of Ornithorhynchus, a monotreme, the most primitive type of mammal showing many affinities with birds and reptiles which is provided with a cartilaginous cup posteriorly.

The animals used for this study were: fish, Fundulus chrysotus; amphibian, Rana catesbiana; reptile, Pseudemys elegans; bird, Gallus sp. (domestic chicken) and Lepus sp., the common laboratory rabbit was used for the mammal.

The term insertion is considered to mean the point at which the tendon fibers meet and merge with the collagenous fibers of the sclera. Tendon is defined as the argyrophilic fibrillae which intervene between the muscle fiber and the collagenous elements of its insertion. I am aware that the classical definition for tendon states that it is composed of collagen, but in no instance have I been able to find an individual muscle fiber which was attached to the sclera except through the intervention of reticulum.

In order to demonstrate the existence of a limiting membrane at the end of the muscle fiber, it is essential that the plane of the section should pass through, or nearly through, the exact center of the longitudinal axis of the fiber. The thickness of the sections is also a very important factor contributing to artefacts because the extreme transparency of the sarcoplasm permits the tendon fibrillae to be seen through the upper levels of tissue. Another source of
error pointed out by Baldwin\textsuperscript{2} is the difficulty of interpreting the morphological details in the case of fibers whose terminations taper to a point. The argyrophilic fibrillae attach all around and for the full length of the cone-shaped tip, so that they form a layer of fibrils which obscures the extreme end of the muscle fiber. The same thing is true of the other types of terminations but to a less extent.

Photomicrographs are appended to this paper in order that the reader may visualise and compare the results achieved by the various technical procedures. However, some of the finer details visible with the microscope are lost. These reproductions are offered only as samples, the conclusions were reached after a study of many serial sections stained by several methods.

Figure 1 (fish) this picture was taken from a section stained by the combined reticulum-connective tissue method and shows the tendon fibrillae of two muscle fibers as wavy black threads which interlace with the collagenous fibers of the solera. The muscle fibers are twisted around each other and at the angle of the curve the reticulum network is evident. The cross-striations extend to the extreme tip of the fiber. In this specimen the fibrous portion of the sclera

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\textsuperscript{2} Baldwin, op. cit.
is very thin and can be seen firmly attached to the relatively large plaque of cartilage.

Figure 2 (frog) shows the tapering end of a single muscle fiber which inserts into the sclera by fine argyrophilic fibrils. The entire fiber, including the tip, is outlined by a thin layer of silver-staining material. On the sides of the fiber this layer appears as a fringe of fine fibrils cut at right angles to their long axes. At the tip of the muscle fiber these fibrils assume a course parallel to the long axis of the muscle fiber and interlace with the collagenous fibers of the sclera. The myofibrillae are evident but show no affinity for the silver nitrate. A spindle-shaped nucleus can be discerned near the end of the muscle fiber among, and closely applied to the tendon fibrillae. Baldwin's² figures 5 and 6 illustrate cells among the tendon fibrillae which he calls fibroblasts. I have frequently observed similar cells in my own preparations (figure 2). Whether these cells are fibroblasts or fibrocytes I am unable to say, but they appear to be closely related to the tendon fibrillae. In most cases the tendon fibrils appear to traverse their cytoplasm.

The muscle fibers pictured in figure 3 conform to the truncated termination pictured by Goss.⁴ The cross-striations

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2 Baldwin, Ibid.
4 Goss, op. cit.
can be seen clearly except at the extreme tip of the fibers. In several places the reticular net has been torn from the muscle fibers and the sarcous portion is left without a membranous covering.

Figure 4 is from the serial group adjacent to figure 3 and received the same treatment except for the omission of silver nitrate. This method fails to show a limiting membrane between the end of the muscle fiber and the tendon. It is comparable to Butcher's figure 14 which he offers as proof of the continuity of myofibrillae with tendon fibrillae. Comparison of this picture with figure 3 shows clearly that the structure described as a transparent protoplasmic membrane, or sarcolemma, is represented by argyrophilic fibrillae when stained by silver nitrate.

Each of the two muscle fibers shown in figure 5 are surrounded by reticular fibrillae which form an anastomosing network uniting the muscle fibers. The argyrophilic net has been torn from the innermost fiber at one point and has taken the sarcolemma with it. The outermost fiber was cut in a slightly tangential plane and reveals the reticular net to be composed of spirally arranged fibrils.

Figure 6 (turtle) shows one muscle fiber cut through the center of the longitudinal axis and several fibers cut in cross-section above. This section was stained by silver

9 Butcher, op. cit.
nitrate without a connective tissue counter-stain. The tendon fibrillae can be seen as black threads attached to the tip of the muscle fiber. These fibrillae unite to form a coarse tendon fiber which extends to the sclera where it mingles with the collagenous fibers. By this method the scleral fibers stain lavender in contrast to the black of the argyrophilic elements. According to the definition used in this paper for tendon the argentofibrils (black) which attach the muscle fiber to the sclera form the true tendon in this case.

Figure 7 was taken from the serial section adjacent to the preceding but was stained lightly by silver nitrate and counter-stained for connective tissue. Although too much of the silver was removed in the ammonium bath, this section is very informative. A comparison with figure 6 emphasizes the affinity of the tendon fibrillae for the silver nitrate. In figure 7 it is impossible to distinguish a limiting membrane at the end of the muscle fiber, so that the myofibrillae appear to fuse in groups and to continue into the insertion as tendon fibrillae. However, these same tendon fibrillae can be distinctly seen to terminate at the external surface of the muscle fiber in figure 6.

The muscle-tendon relationship in the rabbit as shown in figure 8 is seen to be essentially the same as in all the other animals used in this study. This picture was selected to show the intervention of argyrophilic fibrillae
between the muscle fiber and its insertion.

Figure 9 (chicken) is from a tangential section of several muscle fibers stained by the reticulum-connective tissue method. The reticular net surrounding each fiber is very distinct. The sarcoplasm, which is stained red, shows no argyrophilic inclusions.

Verhoeff's elastic tissue method was used on the section of muscle from the chicken pictured in figure 10. This section is representative of all the specimens stained by this method and indicates the absence of elastic tissue. One interesting feature about this section is the entire absence of stain in the tendon and in the reticular and collagenous tissue of the endomysium and perimysium. When this section was compared with an adjacent serial section stained by the combined silver nitrate and connective tissue method it was evident that these structures were reticular in nature.

Discussion

In this investigation I have attempted to secure additional information on several aspects of the attachment of striated muscle fibers.

(1) Is the relation of muscle fibrillae to tendon fibrillae the same in the extrinsic eye muscles of all classes of vertebrates?
In the selection of photomicrographs to accompany this paper an attempt was made to choose examples that illustrate the condition of the muscle-tendon relationship found in all the classes of vertebrates studied. Figures 1, 2, 6, 8 and 9 are from fish, frog, turtle, chicken and rabbit respectively, and reveal the relation of the muscle fibrillae to tendon fibrillae to be essentially the same in all these forms. Figure 1, from a fish clearly demonstrates the intervention of a wavy band of argyrophilic fibrillae between the muscle fibers and the collagenous fibers of the sclera. In figures 2 and 6 the argentofibrils can be seen surrounding the ends of the muscle fibers in the same pattern of attachment. The structural details exhibited in figures 2 (frog) and 9 (rabbit) are so nearly alike that the utmost care had to be exercised in order to prevent interchanging the slides and photomicrographs.

(2) Does the relationship of the myofibrillae to tendon fibrillae in these muscles differ from that described for other muscles?

Goss\(^4\) described the morphology of skeletal muscle attachment in the adult rhesus monkey and states that all the attachments encountered in his investigation showed variations within a general pattern and he felt that further study would

\(^4\) Goss, op. cit.
disclose this scheme to be universal throughout the vertebrates. Since the chief morphological details of the extra-ocular muscles described and pictured here agree with those published by Goss for other muscles, I feel confident that the muscle-tendon attachment is similar.

Baldwin, however, makes a distinction between the condition found in the extrinsic eye muscles of the chick and mouse and those found in the thigh muscle of an adult white mouse. He states, "The figure (#5) demonstrates five muscle fibrillae (A), which proceed directly up to the sarcolemma without losing their features of cross-striation, such as was the case observed with the mouse and chicken extrinsic eye muscles. The tendon end of the sarcolemma is very noticeably thickened and present upon its internal surface small elevations upon each of which a muscle fibril is inserted."^2

In the first place, he is comparing the muscle fibers of an immature animal with those of an adult animal, whereas I have used the extrinsic eye muscles from only adult animals. Fibers of both types can be found in the same muscle and in the extrinsic eye muscles of all the vertebrates which I have studied. Figure 2 (frog) shows the myofibrillae extending to the extreme tip of the muscle fiber, while in figure 7 (turtle) the myofibrillae lose their features of

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^2 Baldwin, op. cit.
cross-striation at the tip of the muscle fiber which appears as an area of structureless sarcoplasm.

From my own observations I am inclined to believe that there is a universal pattern of attachment of the striated muscle fiber to its tendon and that this pattern is expressed in various forms of a reticular net which condenses around the individual muscle fibers during development and fuses at the end to form a large tendon fiber. Also that the variations are correlated with the type of termination of the muscle fiber.

(3) Are the myofibrillae continuous with the tendon fibrillae in the extrinsic eye muscles of vertebrates?

One of the most convincing pieces of evidence on the continuity of myofibrillae with tendon fibrillae that I have encountered in the literature is the discussion of sarcolemma processes given by Baldwin. He states, "The sarcolemma is very thin and is seen in the figure to be drawn out into a number of cone-shaped processes. Into each of these prolongations as many as from 10 to 40 muscle fibrillae enter and, without suffering any reduction in diameter or losing their features of cross-striation proceed directly up to the internal surface of the sarcolemma with which they fuse. To each one of these cone-shaped sarcolemma processes a single tendon fibril is attached.² His figures 6, 7 and 11 make this point

² Ibid.
clear. I have been unable to count the myofibrillae in my preparations but I have tried to demonstrate this condition by contrasting sections stained by silver nitrate with and without a connective tissue counter-stain. Figure 6, stained by the silver component alone, shows the tendon fibrillae attached to the end of a muscle fiber. Only about 6 or 7 of these fibrils are visible, however, figure 7, which is the adjacent serial section stained by the connective tissue component, brings into view the myofibrillae and reveals them to be more numerous. The myofibrillae are also very prominent in figure 2 and, as Baldwin observed, do not lose their features of cross-striation nor suffer any reduction in size. It is difficult to visualize the continuity of from ten to forty fibrils into one of equal size without some reduction in size.

Another bit of conclusive evidence in favor of a limiting membrane between the muscle fiber and the tendon fibrillae is shown in figures 2 and 3 which reveal the terminations of these muscle fibers to be entirely enclosed by a layer of argyrophilic material. At present the selectivity of silver nitrate for reticulum is generally conceded. Goss⁴ and Long⁵ have established the argyrophilic

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4 Goss, op. cit.
5 Long, op. cit.
nature of the tendon fibrillae. Therefore, with this in mind, an examination of figures 2 and 3 will show that no argyrophilic material can be seen inside the sarcous portion of the muscle fiber.

The continuity of myofibrillae with tendon fibrillae has been claimed by Carr in his study of the histogenesis of muscles of the fetal pig. Carr's figure 4 is a camera-lucida drawing of the section of muscle in his figure 3, which was stained by iron hematoxylin and acid fuchsin. He claims that the thinness and proper orientation of his section rules out most of the possibilities for mistaken conceptions advanced by opponents of the continuity theory, such as errors in focusing, superposition of tendon fibrils, mistaking duplications of the sarcolemma for tendon fibrils, etc. But he continues, "It does not rule out the possibility that the fibrils are separated by an extremely delicate sarcolemma which cannot be seen because of destaining, or because contraction following fixation caused its rupture."  

I wish to show by my pictures 2, 3, 6 and 7, that there has been no rupture of the limiting membrane at the tip of the muscle fiber. In figure 6 no attempt was made to stain or demonstrate the sarcolemma as such, only to stain the tendon fibrillae and to establish the fact that these silver-impregnated fibrils are not visible beyond the external

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8 Carr, op. cit.
8 Ibid.
surface of the muscle fiber. On the other hand, in figure 2 the muscle fiber is plainly delimited from the tendon by a network of reticular fibrils. Carr's figure 3 gives no information about the crucial point of interest, namely, the junction between the myofibrillae and the tendon fibrillae. I am inclined to believe that the use of a silver stain would have filled in the details of his sections.

Butcher in his study of the striate muscle of the fetal rat also supports the continuity theory. In the discussion of his methods he lists Mallory's triple stain, Foot's modification of Bielschowsky's method and several others. However, he does not state which method was used on the section reproduced in his figure 14 and offered as proof of the continuity of myofibrillae with tendon fibrillae in a 15-day rat's tail. I realize that I am again referring to observations made on an immature animal and comparing them with mine which are concerned with adult animals, but the similarity between Butcher's figure 14 and my figure 3 is too tempting to resist. The latter, which was stained by the connective tissue component without silver nitrate, shows a similar lack of structural details, so that I would suspect that he also used one of the connective tissue methods which do not reveal the true nature of reticulum. Many

9 Butcher, op. cit.
authors have described a "transition" area at the muscle tip and it is easy to understand this interpretation if one is limited to stains of this kind.

Glücksmann\textsuperscript{14} was particularly interested in the subject from the standpoint of ontogeny and phylogeny. He believes that there are three types of muscle cells which are associated chronologically and functionally with successive types of skeletons (chorda, cartilaginous and bone). The muscle-cell types are: type 1, unicellular; type 2, arising by modification of type 1 through association of connective tissue and blood vessels continuous with the cartilaginous skeleton; type 3, multicellular, arising through sarcomyotic modification of type 2. Phylogenetically the first type appears in Amphioxus. The second type appears in the Selachii and the Teleostei in association with type three. The third type occurs as the persistent stage in all vertebrates beginning with amphibians. Glücksmann states that his research favors the view that the myofibrillae are continuous with the tendon fibrillae.

The mode of attachment of skeletal muscles in the invertebrates has also been a matter of dispute for many

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years. Downey used material from the crayfish and concluded that a definite limiting membrane, the sarcolemma, intervenes between the muscle and the tendon. However, he agrees with Schultze that the reverse is true for the vertebrates. Jordan, on the other hand, investigated the condition in scorpions and added his support to the continuity theory.

My own experience is based on various combinations of silver nitrate and a connective tissue counter-stain and in no instance was I able to observe the insertion of a striate muscle fiber except through the intervention of argyrophilic fibrillae.

(4) Can the sarcolemma be demonstrated as a membrane distinct from the argyrophilic network surrounding each muscle fiber?

The most painstaking search of many serial sections failed to disclose even one instance in which the layer of argyrophilic fibrillae surrounding the individual muscle fibers had been detached from the sarcolemma. Many areas were found where artificial separation of the silver-stained layer had occurred due to preparation but in each instance the sarcous

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11 Downey, op. cit.
6 Schultze, op. cit.
12 Jordan, op. cit.
portion of the muscle fiber was left uncovered by a protoplasmic membrane. The sarcolemma is usually defined as a thin, transparent protoplasmic membrane which encloses the sarcoplasm. Haggquist believes it to be collagenous in nature and to merge with the endomysium. Goss, however, disagrees with this and supports the view that the classical sarcolemma is a combination of a thin protoplasmic membrane and a reinforcing lamella of very delicate argentofibrillae which is intimately adherent to the entire surface of the fiber. However, he states that he prefers to keep the argyrophil fibrillae and collagenous bundles distinct and to retain the individuality of the sarcolemma.

Nagel investigated the relationship of the perimysium internum to the skeletal muscle fiber by means of selective stains including silver impregnation, and the physical nature of the sarcolemma by microdissection. He believes the accessory collagenous bundles of the perimysium internum pass over gradually into a fibrillar reticulum and are distributed spirally on the sarcolemma. In referring to the association of reticulum and sarcolemma, he says, "The reticular network

3 Haggquist, op. cit.
4 Goss, op. cit.
of the sarcolemma is embedded in a homogenous interstitial substance. When explored with a micro-manipulator the sarcolemma behaves as a highly elastic rubber membrane. A comparison of a maximally extended with a totally contracted fiber indicates that the reticular fibers like the membrane itself are highly extensible. The perimysium internum and the sarcolemma forms a tensile-elastic system which is intimately associated with the changes in muscle form during activity."

Jordan\textsuperscript{12} also expresses a belief in the intimate union between the sarcolemma and the inter-fiber connective tissue meshwork in the scorpion.

My observations lead me to believe that the reticular network surrounding each muscle fiber is very firmly joined to the sarcolemma. I feel that the "reinforcing lamella" of Goss, and the "perimysium internum" of Nagel, become more than adherent to the sarcolemma in the adult muscle fiber; the two become fused into a single layer.

(5) Are other tissues such as elastic tissue, concerned in the muscle-tendon attachment of eye muscles?

Many authors have stated that the amount of elastic tissue in the extrinsic eye muscles is quite extensive. Duke-Elder bases his statement on the work of Schifferdecker (1905)
and says, "The amount of elastic tissue in the perimysium and in the interfascicular septa is also quite unusual, a circumstance which seems to be responsible for the passive contraction which occurs after extension by the antagonistic muscle, and which permits a delicate regulation of the movements of the eye." Wolff also quotes the same statement from Schifferdecker. Maximow and Bloom do not give a definite authority for their opinion.

While my problem did not include the elastic tissue content of the extrinsic eye muscles, I wished to investigate every avenue which might add information to the muscle-tendon relationship. With this in mind I stained samples from my material by Verhoeff's method for elastic tissue. The internal elastic lamina of blood vessels was used as a control for the differentiation. The results were uniformly negative in all the specimens. Since samples studied were not considered comprehensive enough to justify a definite opinion, further work is planned on this aspect of the problem.


CONCLUSIONS

(1) The morphology of the muscle attachment of the extrinsic eye muscles of fish, amphibia, reptiles, birds and mammals is essentially the same and is similar to the attachment of skeletal muscle in other parts of the body.

(2) The muscle fiber is completely enclosed by an argyrophilic network which can not be differentiated from the sarcolemma in silver preparations.

(3) In no instance was the insertion of a muscle fiber observed except through the intervention of reticular fibrillae.

(4) The elastic tissue content of the extrinsic eye muscles appears to be less than that usually described in the literature.

Approved 7/23/48

Russell L. Holman
Chairman of Committee
BIBLIOGRAPHY


Figure 1 (fish) Reticulum-connective tissue stain.
The tendon fibrillae of the two muscle fibers shown in this picture unite to form a wavy band which inserts into the thin layer of collagenous tissue surrounding the cartilage plaque.
Figure 2 (frog) Reticulum-connective tissue stain.
The tip of the muscle fiber is completely enclosed by an argyrophilic sheath. A fibrocyte can be seen among the tendon fibrillae.
Figure 3 (frog) Reticulum-connective tissue stain.
The ends of two truncated muscle fibers can be seen to be completely enclosed by argyrophilic fibrillae. Artificial separation of the reticular net and sarcolemma can be seen in several places.
Figure 4 (frog) Connective tissue stain.

This is the adjacent serial section of the muscle fibers shown in Fig. 3. The myofibrillae appear to be continuous with the tendon fibrillae. The argyrophilic network surrounding each fiber seen in the preceding section appears here as a transparent protoplasmic membrane.
Figure 5 (frog) Reticulum-connective tissue stain. Section through the longitudinal axis of two muscle fibers showing the reticular network surrounding each fiber. The reticular net and sarcolemma have been torn from the fiber in one place.
Figure 6 (turtle) Silver nitrate.
The picture demonstrates the argyrophilic nature of the tendon fibrillae and the fibrillar network surround the muscle fibers.
Figure 7 (turtle) Reticulum-connective tissue stain. This picture was taken from the section adjacent to the preceding figure. The myofibrillae appear to fuse in groups and continue into the insertion as tendon fibrillae.
Figure 8 (rabbit) Reticulum-connective tissue stain. This figure is similar to Fig. 2 (frog) and shows that the muscle-tendon relationship is essentially the same in the two animals.
Figure 9 (chicken) Reticulum-connective tissue stain. Tangential section through several muscle fibers showing the argyrophilic network of fibrils.
Figure 10 (chicken) Verhoeff's elastic tissue stain.
This picture is representative of all the specimens stained by this method and shows the absence of elastic tissue.
EXAMINATION AND THESIS REPORT

Candidate: Miss Aeleta Barber

Major Field: Zoology

Title of Thesis: A comparative study of the extrinsic muscle attachment of the eye of vertebrates.

Approved:

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Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination: 7-1-48