The demonstration of different types of muscle fibers in human extraocular muscle by electron microscopy and cholinesterase staining

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Surgical specimens of normal human extraocular muscle revealed in electron microscopic cross sections two distinct types of extrafusal fibers: (1) a fibrillar type (Fibrillenstruktur) and (2) an essentially afibrillar type (Felderstruktur). Fascicles of autopsy muscle, stained with a modified Koelle technique to demonstrate cholinesterase activity, showed by light microscopy two kinds of nerve endings: (1) large, heavily staining, compact discs (typical motor end plates or "en plaque" endings), which usually occurred singly within the distance teased, and (2) smaller, lighter staining droplets in clusters or chains ("en grappe" endings), which occurred multiply on a single fiber. The two nerve terminal types were never seen together on the same muscle fiber. Differential staining, with selected substrates and an inhibitor (DFP), showed the presence of both acetyl and butyryl cholinesterases in each ending. Electron microscopy of muscle stained for cholinesterase correlated the fibrillar ultrastructure with the fibers possessing "en plaque" endings and the afibrillar ultrastructure with the fibers possessing "en grappe" endings. These morphologic features strongly suggest that human extrinsic eye musculature is organized into two separate contractile systems similar, if not identical, to the fast and slow striated muscle systems conclusively established in the frog. A brief discussion of the oculomotor implications is included.

The existence of separate fast and slow contractile systems in amphibians was suggested in 1928 by the physiologic studies of Sommerkamp1 on the "tonus bundle" of frog iliofibularis muscle. Krüger2 and Fürlinger3 described two kinds of skeletal muscle fibers based on distinct histologic and innervational differences between fibers located outside this "tonus bundle" and many of those present within. These findings were confirmed in a variety of frog muscles and extended to other submammalian vertebrates by Krüger and his co-workers,4, 5 and in this country by Hess,6-8 who applied a modified Koelle-Friedenwald cholinesterase stain to characterize further the innervation of these two fiber types. These morphologic fiber types, named Fibrillenstruktur and Felderstruktur by Krüger, can be most easily distinguished in transverse

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sections of muscle, and are based on myofibrillar shape and size. Fibers exhibiting Fibrillenstruktur have small, well-delineated fibrils, each surrounded by abundant sarcoplasm, giving an even punctate appearance in the light microscope. Furthermore, these fibers, when examined following nerve or cholinesterase stains, demonstrate single, large, compact "en plaque" (plaque-like) nerve endings (typical motor end plates), terminating large diameter motor nerves. Fibers are said to possess Felderstruktur when they contain large blocks of poorly delineated fibrils (almost a mass of myofilaments) as a result of meager separation of fibrils by sarcoplasm, as seen in cross sections. These essentially afibrillar fibers are provided with numerous, small, "en grappe" (grapelike) nerve endings arranged linearly or in loose collections. These endings are derived from efferent nerves of small diameter. Krüger and his associates believed their investigations indicated two separate contractile mechanisms possessing independent innervation to be the basis for the two responses. Fibrillenstruktur fibers were responsible for the fast, twitch, or phasic reaction, while Felderstruktur fibers were capable only of slow, tonic, or acetylcholine contractions. Conclusive physiologic evidence by Kuffler and Vaughan Williams, electron microscopic verification of these two distinct muscle fiber types (fibrillar with an elaborate sarcoplasmic reticulum and afibrillar without an extensive reticulum) by Edwards and co-workers, and direct correlation of functional response with ultrastructural type by Peachey and Huxley have clearly established the existence of separate extrafusal, fast and slow neuromuscular systems among submammalian vertebrates.

Krüger and his co-workers have insisted that a similar system existed in mammals, not only in the extracocular muscles, but, for example, in the diaphragm and soleus as well. Most workers, however, such as Hess, are convinced that only within the extrinsic eye muscles do mammals possess the twitch and tonic systems of lower forms. Although reports of the presence of grapelike endings on extracocular muscle date from Retzius in 1892, and extensive investigations of eye muscle innervation have continued to the present, little attention in ophthalmic literature has been directed toward these two complementary contractile systems, in spite of the clear evidence of Hess as to their presence, both anatomically and physiologically, in subhuman species. Recent papers by Kupfer, Cheng, and Wolter applying combined cholinesterase and nerve fiber stains to frozen sections of human eye muscle, omitted any direct reference to the tonic striated system, which would correlate their findings of small grape-like endings with data regarding the structure and innervation of mammalian extraocular muscle.

In the present investigation, the morphologic techniques used by Hess were applied to human extrinsic eye muscles, with the intention of demonstrating the presence or absence of the two fibril types. This would be significant in any future formulation of oculomotor function in man.

Materials and methods

Normal, adult, human striated muscle was obtained from each of the six extracocular muscles at surgical enucleation and at postmortem examination of individuals who had died within the previous 18 hours. The levator palpebrae was included in the autopsy material.

The surgical specimens, limited to the distal 3 to 4 mm., were utilized for routine electron microscopy and provided adequate tissue for examination in all muscles except the superior oblique. Each muscle portion was immersed and cut into 1 mm. blocks in cold 1 per cent osmium tetroxide in Veronal acetate buffer (pH = 7.4 to 7.6), according to Palade, containing 0.03 per cent calcium chloride and 8.2 per cent sucrose. After 1.5 hours' fixation the tissue was washed briefly in ice cold saline, dehydrated in graded 10 per cent steps of ethanol (10 minutes each), and stored until needed in warm tertiary butyl alcohol. Several specimens were treated initially with cold 5 per cent glutaraldehyde in 0.2 M sodium cacodylate buffer (pH = 7.45). After 4 hours' fixation the tissue was washed for 2 hours.

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in cold buffer, transferred to 1 per cent osmium tetraoxide, and handled as outlined above. The glutaraldehyde and wash used in this alternate procedure contained added calcium and sucrose identical to the osmium fixative. Following an hour in toluene, the muscle was embedded in Araldite, and polymerized in a 60°C oven overnight. Sections with a silver interference color, cut with glass knives on an LKB Ultramicrotome, were collected on naked grids, stained with lead or uranium acetate, and photographed in an RCA EMU-2E electron microscope.

To insure that the conclusions reached were derived from extrafusal fibers, and to facilitate the identification of Felderstruktur, each block was oriented for transverse section and studied initially with thick sections with the use of phase contrast optics. Muscle spindles, though infrequent in the distal area under study, were easily identified as two to six muscle cross sections encased in a thin capsule, quite similar to those in the pictures and descriptions of Cooper and Daniel. Such structures, when isolated and thin sectioned, yielded a cross-sectional morphology in agreement with the ultrastructural findings of Merrillers. At no time were conclusions drawn from any fiber which in the phase or electron microscope gave evidence of incorporation into a spindle capsule.

The autopsy material, consisting of intact muscle and teased preparations, was employed to complete the above electron microscopic survey (i.e., it was the sole source of the superior oblique) and also for conducting the following cholinesterase studies. Following 3 per cent, ice cold, glyoxal fixation for 2 hours in an extended state, a modified Koelle and Friedenwald cholinesterase technique was applied in three groups of experiments. First, a routine stain was carried out upon each whole muscle and on small fascicles teased from origin to insertion. In this procedure there was a one-hour incubation at room temperature with acetylthiocholine substrate (pH = 4.8). Following exposure to 2.2 per cent ammonium sul-
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fide, the tissue was returned to 3 per cent glyoxal overnight for completion of the fixation. The whole muscle preparations were examined under fixative in a dissecting microscope for orientation and a gross impression of innervation. The small fascicles were further teased under glycerine into groups of one to five fibers. The lengths obtained were no greater than 5 mm. because of the friability of the tissue. Such preparations were examined in a light microscope for structure and distribution of endings. Although all areas of a fascicle were examined, conclusions regarding extrafusal innervation were derived from fibers within the middle third of the muscle, avoiding the proximal and distal thirds, wherein the spindles are located. The levator was used in this study as a control.

To investigate the pharmacology of these nerve endings, teased portions of the lateral rectus muscle were incubated 2 hours in either acetylthiocholine (ATCh) or butyrylthiocholine (BTCh) with other conditions as before. Portions requiring a butyryl cholinesterase inhibitor were immersed in Floropryl* (0.1 per cent diisopropyl fluorophosphate [DFP] in peanut oil) for 60 minutes and washed in saline for 5 minutes before exposure to the substrate. Table I indicates the combinations employed for enzyme differentiation. Finally, to correlate the type of ending with ultrastructural morphology, teased fascicles of the inferior oblique were stained for cholinesterase and divided under glycerine into a group of fibers possessing mainly "en grappe" endings and another mainly "en plaque" endings. (It was found most difficult to tease out bundles entirely free of one or the other ending and still be grossly visible for handling.) Each group was then washed in saline and handled for routine electron microscopy, as outlined.

Results

The observations described below (unless previously stated otherwise) were made on each of the rectus and oblique muscles and were in every case identical.

**Extrafusal muscle fibers.** Examination of transverse sections of muscle, at the preliminary phase microscopic level, provided two contrasting muscle fiber cross sections. One type, conforming to descriptions in the literature of Fibrillenstruktur fibers, yielded a fine, uniform, stippled appearance with seemingly even spacing of the component myofibrils (Fig. 2, Fb). The other, possessing Felderstruktur characteristics, gave a coarse, clumped, or broken profile with an irregular and haphazard arrangement of larger fiber components (Fig. 2, Fl). These two fiber types were observed randomly and approximately equally throughout each section studied.

Examination in the electron microscope of such cross sections, regardless of the fixation procedure employed, confirmed the existence of two distinctly different muscle fibers. The finely punctate, uniform fibers demonstrated small, discrete myofilaments, clearly delineated by an abundant sarcoplasm with its reticular and particulate elements (Figs. 3 and 4). Fibers having a globular, clumped structure were found organized into a more or less afibrillar mass of myofilaments with large, poorly defined, partially fusing fibrils in a sparse sarcoplasm containing an underdeveloped sarcoplasmic reticulum (Figs. 5 and 6). These ultrastructural differences correspond closely to those confirmed by Peachey and Huxley in fibers of frog muscle having twitch and slow responses, which they isolated and classified physiologically and then examined by electron microscopy.

**Extrafusal nerve endings.** When observed in a 20× dissecting microscope, the whole preparations of cholinesterase-stained extraocular muscle displayed two striking features. There was readily apparent a darkly staining, irregular band (1 to 2 mm. wide) which encircled the muscle and lay approximately in the middle third somewhat distal to the point of union of the muscle and its nerve trunk. This area, corresponding to the terminal innervation band, is formed by staining of the "en plaque" end-

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*Courtesy of Merck Sharp & Dohme, Philadelphia, Pa.

Table I. Combinations employed for enzyme differentiation

<table>
<thead>
<tr>
<th>DFP</th>
<th>Substrate</th>
<th>Enzyme demonstrated</th>
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<tbody>
<tr>
<td>0%</td>
<td>ATCh</td>
<td>AChE and BChE</td>
</tr>
<tr>
<td>0.1%, 60 minutes</td>
<td>ATCh</td>
<td>AChE</td>
</tr>
<tr>
<td>0</td>
<td>BTCh</td>
<td>BChE</td>
</tr>
<tr>
<td>0.1%, 60 minutes</td>
<td>BTCh</td>
<td>Neither (control)</td>
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*Courtesy of Merck Sharp & Dohme, Philadelphia, Pa.*
Fig. 3. Fibrillenstruktur, slightly tangential section. Selected area within a single muscle fiber demonstrating small discrete myofibrils (f). Mitochondrion (m); sarcoplasmic reticulum (sr). (Surgical specimen, 1 per cent osmium tetroxide, electron micrograph, x20,800.)

Fig. 4. Fibrillenstruktur, transverse section, similar to that in Fig. 3. Note the well-delineated myofibrils (f) with their component myofilaments (arrows) clearly visible in cross section. Mitochondrion (m); sarcoplasmic reticulum (sr). (Surgical specimen, 5 per cent glutaraldehyde followed by 1 per cent osmium tetroxide, electron micrograph. x20,800.)
Fig. 5. Felderstruktur, transverse section. Selected area within a single muscle fiber revealing large indistinct myofibrils (f) with the component myofilaments (arrows) blending into a continuous mass. Mitochondrion (m); sarcoplasmic reticulum (sr). (Surgical specimen, 1 per cent osmium tetroxide, electron micrograph. x20,800.)

Fig. 6. Felderstruktur, transverse section, similar to that in Fig. 5. Large, poorly defined myofibrils (f); myofilaments (arrows); mitochondrion (m); sarcoplasmic reticulum (sr)—the membranes are less obvious than those in Fig. 5. (Surgical specimen, 5 per cent glutaraldehyde followed by 1 per cent osmium tetroxide, electron micrograph. x20,800.)
Fig. 7. Fibrillenstruktur, transverse section. Selected area within a single muscle fiber representative of the vast majority in a "mainly en plaque fiber" group. The preponderance of the relatively discrete myofibrillar pattern (f) in a bundle of fibers possessing mostly "en plaque" endings is quite suggestive that these two characteristics occur together and define a particular muscle fiber type. Myofilaments (arrows); mitochondrion (m); sarcoplasmic reticulum (sr). (Autopsy specimen, cholinesterase stained, and nerve endings identified, 1 per cent osmium tetroxide, electron micrograph. x20,800.)

ings and is found in all striated muscle. In addition, a peculiar speckled staining was evident over the entire muscle surface from origin to insertion, suggesting a diffuse widespread innervation as well. In marked contrast, the levator palpebrae muscle displayed only the dense focal band of end plates.

At higher magnification, teased extraocular fibers displayed two kinds of stained terminals. One was a large, intensely staining, compact, often lobulated, oval ending with an appearance quite similar to the "en plaque" ending of the classical fast or twitch fiber (Figs. 9, 10 to 12). The second type appeared as smaller, lighter staining, droplets, buttons, or beads organized in loose clusters or attached chains, identical to the "en grappe" endings associated in other species with the slow contractile response (Figs. 13 to 16). The levator possessed only "en plaque" innervation.

The distribution of each type of ending was characteristic. Although entire lengths were not observed, the two nerve terminal classes never could be found on the same muscle fiber. This situation suggested two muscle fiber types: (1) those with motor end plates and (2) those with grapelike endings. "En plaque" terminals usually occurred singly on individual fibers, both within the terminal innervation band and when encountered elsewhere. However, two "en plaque" endings were occasionally seen within the distances teased (Fig. 12). Since it was possible to find these endings scattered throughout the muscle from origin to insertion, it was felt that several widely spread "en plaque" terminals might be present on single fibers. This conclusion is supported by the work of Kupfer and Cheng. In contrast, numerous "en grappe" endings were present within any teased length (Figs. 13 to 16). In agreement with
Fig. 8. Felderstruktur, transverse section. Selected area within a single muscle fiber representative of the vast majority in a “mainly en grappe fiber” group. The preponderance of this indistinct myofibrillar pattern (f) in a bundle of fibers possessing mostly “en grappe” terminals supports the belief that these two features also occur simultaneously, and hence establish a second muscle fiber type. Myofilaments (arrows); mitochondrion (m); sarcoplasmic reticulum (sr). (Autopsy specimen, cholinesterase stained, and nerve endings identified, 1 per cent osmium tetroxide, electron micrograph. x20,800.)

Table II. Results of differential staining

<table>
<thead>
<tr>
<th>DFP</th>
<th>Substrate</th>
<th>Enzyme demonstrated</th>
<th>“En plaque” endings</th>
<th>“En grappe” endings</th>
<th>Reaction intensity</th>
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<td>0</td>
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<td>AChE, BChE</td>
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<td>+ (Fig. 17)</td>
<td>4+</td>
</tr>
<tr>
<td>0.1 %</td>
<td>ATCh</td>
<td>AChE</td>
<td>+ (Fig. 19)</td>
<td></td>
<td>1+</td>
</tr>
<tr>
<td>0.1 %</td>
<td>BTCh</td>
<td>BChE</td>
<td>+ (Fig. 18)</td>
<td>+ (Fig. 20)</td>
<td>1+</td>
</tr>
<tr>
<td>0</td>
<td>BTCh</td>
<td>Neither (control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

Hess's studies in the monkey, great variability was encountered in the distances between “en grappe” endings, both on the same fiber and on different fibers, with extremes in the range of 10 μm to 2 or 3 mm. Isolated fibers of the levator demonstrated single motor end plates in the innervation band.

An investigation of the pharmacology of these two nerve endings was undertaken on only one of the extrinsic eye muscles, since the results enumerated above had indicated each to be identical within the parameters of the current investigation. Haggqvist has postulated recently that “en plaque” endings may contain only acetyl cholinesterase (AChE; true cholinesterase), while “en grappe” endings stain for butyryl cholinesterase (BChE; pseudocholinesterase). Furthermore, he demonstrated in the monkey lateral rectus only the acetyl fraction. The lateral rectus therefore was selected for differential staining, with the results in Table II. Thus, both
Fig. 9. "En plaque" terminal, teased preparation. Selected portion of an isolated muscle fiber (F) demonstrating a single, large, oval motor end plate (arrow). (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. x390.)

Fig. 10. "En plaque" terminal, teased preparation, similar to that in Fig. 9. Another muscle fiber (F) with its compact, platelike motor ending (arrow). (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. x390.)

Fig. 11. "En plaque" terminals, teased preparation similar to those in Fig. 9 displaying single neuromuscular terminals (arrows) on two separate muscle fibers (F). (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. x390.)

Fig. 12. "En plaque" terminals, teased preparation, similar to those in Fig. 9 with the exception that the rare situation of two plaquelike endings (arrows) on a single muscle fiber (F) is demonstrated. Two other superimposed "en plaque" endings are just visible at the upper left. (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. x390.)

Fig. 13. "En grappe" terminals, teased preparation. Selected portion of single muscle fiber (F), which is in marked contrast to those in Figs. 9 to 12. Numerous small drops or beads of stain (arrows) arranged in linear array are easily visible. At no time are these grapelike endings found in a single isolated fashion like "en plaque" endings. (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. x390.)

Fig. 14. "En grappe" terminals, teased preparation, similar to those in Fig. 13. Multiple, linear staining areas (arrows) on a single muscle fiber (F). A branching cluster arrangement seen frequently is noted (C). (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. x390.)
Fig. 15. "En grappe" terminals, teased preparation, similar to those in Fig. 13. Five small grapelike endings (arrows) are visible again on a single fiber (F). (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. ×390.)

Fig. 16. "En grappe" terminals, teased preparation, similar to those in Fig. 13. A single fiber (F) possessing several endings, some of which (C) are in a branching cluster. (Autopsy specimen, acetylthiocholine substrate staining acetyl and butyryl cholinesterase, light micrograph. ×390.)

Introduction to Figs. 17 to 20. These figures demonstrate that "en plaque" as well as "en grappe" nerve endings are stained following the differential procedures (see text) for revealing acetyl and butyryl cholinesterase separately. The decreased reaction intensity evident upon comparison with Figs. 9 through 16 is felt to be the result of selectively staining for only one of the two esterases present. (Hess believe maximal staining takes place only when both enzymes are active.)

Fig. 17. "En plaque" terminal, AChE positive, teased preparation. Selected portion of an isolated muscle fiber (F) treated with DFP and followed by acetylthiocholine substrate, which shows only acetyl cholinesterase. A single, compact, platelike ending (arrow) is seen. (Autopsy specimen, light micrograph. ×390.)

Fig. 18. "En grappe" terminals, AChE positive, teased preparation, similar to those in Fig. 17. A single muscle fiber (F) treated with DFP and followed by acetylthiocholine substrate, which shows only acetyl cholinesterase. Several grapelike endings (arrows) are visible. (Autopsy specimen, light micrograph. ×390.)

Fig. 19. "En plaque" terminal, BChE positive, teased preparation. Selected portion of an isolated muscle fiber (F) treated with butyrylthiocholine substrate, which shows only butyryl cholinesterase. One large, oval end plate (arrow) is visible. (Autopsy specimen, light micrograph. ×390.)

Fig. 20. "En grappe" terminals, BChE positive, teased preparation, similar to those in Fig. 19. A single muscle fiber (F) treated with butyrylthiocholine substrate, which shows only butyryl cholinesterase. Several small, staining areas (arrows) can be seen. (Autopsy specimen, light micrograph. ×390.)
“en plaque” and “en grappe” endings are revealed following each enzymatic reaction. This would suggest, at least at this level of histochemical sensitivity, that the two terminal types are qualitatively similar in enzyme reactions. Hess has demonstrated each cholinesterase in both endings in frog and monkey striated muscle. The diminished reaction intensity seen during differential staining was also described by Hess. He believed that maximal staining took place only when both esterases were active. The use of either DFP followed by acetylcholinesterase, which demonstrates only acetyl cholinesterase (Figs. 17 and 18), or butyrylcholinesterase, which demonstrates only butyryl cholinesterase (Figs. 19 and 20), resulted in fainter staining because only one of the two enzymes present was functioning.

Correlation of muscle ultrastructure with kind of nerve ending. To determine if the muscle fibers with “en plaque” and “en grappe” endings were identical to the fibrillar and afibrillar muscle fiber types, respectively, an electron microscopic examination was made of previously stained fibers from postmortem inferior oblique. One group was designated “mainly en plaque fibers” and the other “mainly en grappe fibers.” Each group, when studied, displayed a vast majority of its fibers to be of one structure or the other. The “en plaque” fiber group possessed primarily Fibrillenstruktur (Fig. 7), while the “en grappe” fiber group demonstrated a preponderance of Felderstruktur (Fig. 8). Although the impurity of the groups prevents exact correlation, these findings suggest that the fibrillar fibers have only “en plaque” endings, and the afibrillar fibers only “en grappe” terminals, which is in agreement with the conclusions of Hess.

Discussion

The presence of both fibrillar (presumptive fast) and afibrillar (presumptive slow) muscle fiber ultrastructure, the demonstration of cholinesterase positive “en plaque” and “en grappe” nerve endings, which exist on separate contractile elements, and the suggestive correlation of each fiber class with an innervational type have provided for human extraocular muscle the same morphologic features as are present in frog iliofibularis muscle, wherein the existence of separate fast and slow contractile mechanisms has been physiologically established. These anatomic findings should provide a strong impetus for the initiation of physiologic studies, such as nerve fiber stimulation associated with intracellular recording and tension measurements, which are clearly required to establish as fact the presence of fast and slow contractile systems in human extraocular musculature. The recent investigation of cat superior oblique muscle by Hess and Pilar, where physiologic as well as anatomic evidence for separate twitch and tonic responses is presented, indicates that functional studies in man will confirm the structural impressions of this report. Anatomic absence of the slow muscle fiber system in the levator of the lid, a muscle also receiving innervation from the oculomotor nerve, can be regarded as evidence for a selective unique organization of the rectus and oblique musculature. The presence of such a system limited precisely to these six related muscles implies a significance greater than a biologic curiosity.

Although conclusive evidence is lacking, the belief that the “en grappe” endings demonstrated in this paper were somatic motor terminals was based primarily on their cholinesterase positive staining, which Coers and Woolf feel (at least in muscle tissue) strongly implies a motor function. The work of Corbin and Oliver demonstrating the cell bodies of origin of the “en grappe” endings in cat extrinsic eye muscle to lie neither in the trigeminal nucleus nor in its mesencephalic root, but within the third, fourth, and sixth cranial nerve nuclei, supports this assumption. Furthermore, these workers, through ablation and retrograde recording, showed that neurons in the superior sympathetic ganglion and
Edinger-Westphal nucleus play no part in supplying “en grappe” innervation. Such data would seem to exclude the possibility of attributing an autonomic function to these endings.

The properties of the small diameter motor nerves, the junctional potential, and the contractile response which characterize the slow muscle system can be found in detail in the papers of Kuffler and Vaughan Williams.\textsuperscript{9, 20} Suffice it to include here that the slow and fast fibers were found synergistic, the state of tension of the slow fibers was directly related to stimulus frequency, and any amount of relaxing slow fiber tension could be collapsed instantly by superimposition of a single twitch contraction. Should such a versatile neuromuscular organization be established for human extrinsic eye muscle, a specific mechanism might be available to explain, for example, the phenomenon of simultaneous horizontal, fast saccadic, and slow vergence movements as described by Alpern and Wolter.\textsuperscript{21}

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