NEUROANATOMICAL STUDIES OF HUMAN EXTRAOCULAR MUSCLES

A Thesis
submitted by

Jan Richard Bruenech

for the degree of
DOCTOR OF PHILOSOPHY

Department of Optometry and Visual Science
The City University
October 1996

CONTENTS

CHAPTER 2

MATERIAL AND METHODS

CHAPTER 3

RESULTS

CHAPTER 4

DISCUSSION

APPENDIX

LIST OF TABLES AND ILLUSTRATIONS

CHAPTER THREE

ACKNOWLEDGEMENT

I wish to express my gratitude to Professor Gordon L. Ruskell for his skilful supervision and for placing his expert anatomical knowledge at my disposal. I am indebted to Professor Martin Steinbach of the Hospital for sick children, Toronto and Mr Kin Wang FRCS of Luton and Dunstable Hospital for providing and undertaking the initial processing of some of the material used in this study. I am also grateful to my colleagues at Buskerud College for their kind assistance during all these years. The financial support provided by Hultgren`s Memorial Foundation and the Norwegian Optometric Society is gratefully acknowledged.

I dedicate this thesis to my family from which most of the time was taken.

DECLARATION TO THE UNIVERSITY LIBRARIAN

I grant powers of discretion to the University Librarian to allow this thesis to be copied in whole or in part without further reference to me. This permission covers only single copies for study purposes, subject to normal conditions of acknowledgement.

ABSTRACT

Detailed knowledge of the neural arrangement in the human extraocular muscles (EOM) is fundamental for the understanding of oculomotor control yet information regarding both efferent and afferent innervation is incomplete. Previous analysis of the nerve fibre distribution and estimates of the motor unit have failed to include the full range of fibre diameters and are subject to criticism. What fraction of these nerves is afferent is still unknown and the role of the sensory receptors is disputed. A variety of features observed in aged extraocular muscle spindles were described recently leading to the contention that they are probably incapable of proprioception. Other mechanoreceptors have been observed in the distal tendon, but whether or not these receptors can be equated with Golgi tendon organs is unclear.

The aims of this study were, therefore, first to analyse the structure and consider the potential function of spindles in infant human EOM and to compare infant muscles with the existing knowledge of adult muscles. Secondly, tendon innervation was also studied over the same age range. Finally, total nerve and muscle fibre counts were made and motor units calculated incorporating a correction factor to allow for the estimated portion of sensory fibres.
Thirty one muscle samples were obtained from 19 patients aged 1 day to 90 years. None of the patients had any history of binocular abnormalities or neuromuscular disease. The samples were fixed in 5 % glutaraldehyde, prepared using standard histological techniques and examined by light and electron microscopy.

Quantitative analysis of the fibre content of 4 inferior oblique muscles displayed a reduction in the number of muscle fibres with age (correlation factor

0.969). The specimen obtained from the oldest subject (90 years), contained only 36% of the number of fibres found in the youngest subject (42 years). Analysis of the nerves presented a trimodal distribution of fibre diameters where the unmyelinated axons constituted a significant portion of the total. It is legitimate to include these fibres in calculation of the motor unit because of their efferent nature, although in earlier estimates this was not done. Felderstruktur fibres, known to be served by such small diameter efferents, have therefore arguably a smaller motor unit than previously assumed.

In contrast to the muscles, the nerves presented insignificant variations in fibre content with age. This resulted in a variation in the size of the motor unit, from 4.75 in the youngest to 1.87 in the oldest subject. These figures were determined from nerve and muscle fibre numbers alone after subtraction of the estimated number of sensory nerve fibres. Estimates of the sensory nerves were based on the content of intrafusal muscle fibres and number of tendon receptors.

Forty spindles, obtained from the distal end of 20 muscle samples, were examined in transverse serial sections . They varied in length from 47 to 683μm (mean 323μm) and contained between 2 and 12 intrafusal muscle fibres. The majority of intrafusal muscle fibres were fragmented and/or failed to run the full length of the spindle. Most spindles had a small periaxial space and contained at least one large fibre lacking a nuclear bag or chain region.

The presence of interrupted intrafusal fibres with their sensory portion disconnected from a contractile pole makes it seem unlikely that any ordered deformation of the primary sensory ending will occur during a muscle contraction. Hence, the ending may be incapable of responding to stretch. Furthermore, unmodified fibres with presumed extrafusal functional characteristics within the spindle would prevent independent action by the spindle. These factors along with other structural departures from the

conventional spindle, previously found in adults and now in infants, give reason to question the muscle spindle's functional capacity.

No sensory receptors were found in the distal tendon in infant material but in adults axon terminals were present in a few areas. Certain of these structures bore mechanoreceptorial features yet none of them was found to be encapsulated or presented typical Golgi tendon organ form. Their introduction in mature muscle is arguably the result of motor end plate lability and of the redundant fibres from spindles seeking new targets.

The absence of tendon receptors in the infant material and the peculiar structure of the spindle suggest that muscle proprioception must be limited, at least in the early stages of life and probably is of questionable functional significance. Earlier proposals that proprioception plays a major role in development and maintenance of binocularity are inconsistent with these findings. These observations add credence to alternative explanations for eye position monitoring, in particular the efference copy hypothesis.

LIST OF SYMBOLS AND ABBREVIATIONS USED IN TEXT

Acetyl cholinesterase	AchE
Charge Coupled Device	CCD
Extraocular muscle/s	EOM
Frontal eye field	FEF
Golgy tendon organ	GTO
Inferior oblique muscle	IOM
Inferior rectus muscle	IRM
Lateral rectus muscle	LRM
Motor endplate	MEP
Multiply innervated fibres with action potentials	MIC
Tonic, multiple innervated fibres without action potentials	MINC
Medial longitudinal fasciculus	MLF
Medial rectus muscle	MRM
Parieto-occipital-temporal cortex	POT
Paramedian pontine reticular formation	PPRF
Superior colliculus	SC
Twitch, single innervated fibres	SI
Sarcoplasmic reticulum	SR
Superior rectus muscle	SRM
Transverse tubulus	t-tube
Vestibular nucleus	VN

CHAPTER 1

INTRODUCTION

1.1 STRUCTURE AND FUNCTION OF THE HUMAN EXTRAOCULAR MUSCLE (EOM).

The extraocular muscles constitute the effector organ of the oculomotor system. In addition to the muscles, the oculomotor system includes the three ocular motor nerves and all supranuclear structures acting upon their neurones. Through observations of pathological conditions in humans and from animal experiments, it has been found that the supranuclear stimulation arises from several neural components located in the brainstem, the cerebellar and cortical systems (for review see Büttner & Büttner-Ennever, 1988). Structures such as the vestibular apparatus, superior colliculus, frontal and occipital areas of the cortex, have neural pathways connecting them with the ocular motor nuclei. They project either directly, through internuclear pathways such as the medial longitudinal fasciculus (MLF) or via immediate premotor structures such as the paramedian pontine reticular formation (PPRF). The sum of stimulation and inhibition from the supranuclear components will dictate the discharge frequency in the motor nerves (Fig. 1.1). Once the motor neurone is stimulated to discharge at a set frequency, the signal cannot be altered before contraction of the receiving muscle fibres has taken place. Any deviation between the desired movement and the one actually being performed can only be adjusted by re - stimulating the muscle in question or its antagonist. This neural arrangement was first observed in skeletal muscle many years ago and is now commonly referred to as "the final common pathway". In the oculomotor system, this pathway has a generous complement of efferent nerve fibres compared to the rather modest number of muscle fibres they supply.

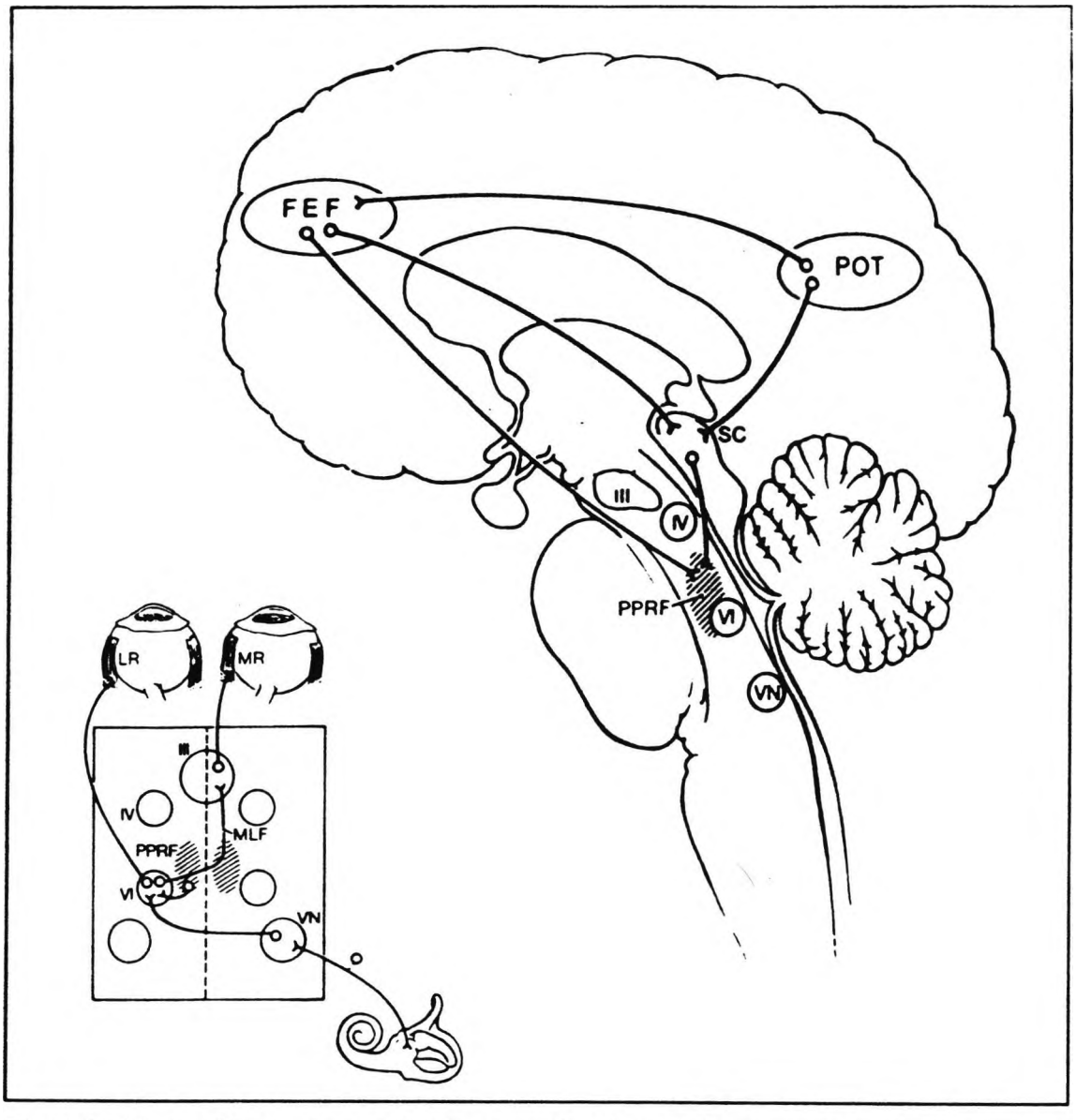


Figure 1.1 The two figures summarise the connections between the supranuclear structures participating in horizontal eye movement control. The top figure shows the supranuclear connections from the frontal eye fields (FEF) and the parieto-occipital-temporal junction region (POT) to the superior colliculus (SC) and the paramedian pontine reticular formation (PPRF). The lower figure shows how axons from the cell bodies located in the PPRF travel to the ipsilateral abducens nucleus where they synapse with abducens motor neurones whose axons travel to the ipsilateral lateral rectus muscle (LRM). These axons also synapse with abducens interneurones whose axons cross the midline and travel in the medial longitudinal fasciculus (MLF) to the portion of the oculomotor nucleus concerned with medial rectus (MRM) function in the contra lateral eye. Axons from the cell bodies located in the vestibular nuclei (VN) travel directly to the abducens nuclei and, mostly via the MLF, to the oculomotor nuclei. (Modified from N.R. Miller. In Walsh and Hoyt's Clinical Neuro-Ophthalmology. Williams & Wilkins, 1985)

The low ratio between muscle and nerve fibres partly accounts for the precise gradations in motility of the eye. The cranial nerves innervating the EOM are classified as somatic motor nerves carrying only one modality (Wilson-Pauwels et al, 1988). They do, however, contain a large complement of afferent fibres as well. Any signal travelling in the sensory nerves must arise from various specialised nerve endings located either within the bulk of the muscle or at the musculotendinous interface. The presence of such receptors suggests that muscle activity can be monitored and readjusted, matching the system of motor control in skeletal muscles.

Development of normal visual function is dependent upon appropriate retinal stimulation. The oculomotor system continuously adjusts the position of the two eyes so that this stimulation remains optimum at all times. Whether the required accuracy of these eye movements arises purely through the elaborate efferent nerve supply or by the assistance of a complex afferent feedback system, is still unknown. There is, however , no apparent reason to doubt that fine motor control of the EOM is essential for both the developmental process and maintenance of binocular vision.

1.1.1 Embryology

The embryology of the extraocular muscles in man is not unique but follows the pattern seen in several species (Eggers, 1982). They develop from condensations of mesoderm that initially appear at the 7 mm stage (size of embryo measured from crown to rump). It is possible to detect the differentiation into recti and oblique muscles at quite early stages and by the 20 mm stage the complete outline of all muscles is usually present in histological light microscopic sections. According to Sevel (1981) the origin, belly and insertion of the muscle develop simultaneously. Furthermore he noted that all the individual muscles develop at the same time.

As with conventional striated muscle tissue, the muscle fibres proliferate from mononucleated cells into mature multinucleated muscle fibres. This development goes through several stages starting with mesenchymal tissue. Mesenchymal cells are widely separated from each other, but together they form a spongy meshwork which can be observed in embryos up to 36 mm in size. They then change into myoblast cells. The myoblast cells undergo continuous development and have, in their initial stages, round or oval nuclei surrounded by a ring of cytoplasm. The absence of conventional muscle cell features makes it difficult to identify early myoblast cells with any certainty (Gamble et al, 1978). When the myoblast cells mature they take up a spindle shape and cross striations can be observed at the 38 mm stage and onwards. In the final stage (54 mm) the myoblast cells fuse to form the multinucleated myotube cells. Cross striations are now present in most cells but displacement of nuclei is yet to occur. At this stage of muscle fibre development, the myotube cell resembles the mature intrafusal muscle fibre with a chain of centrally placed nuclei. By the time the fetus reaches the 80 mm stage, most cells consist of a syncytium of elongated cells with peripherally located subsarcolemmal nuclei (Sevel, 1981). Displacement of nuclei designates the final stages of the developmental sequence (Fig. 1.2).

The muscle fibres appear to differentiate into specific fibre types between 12 and 18 weeks of gestational age, depending on the method of observation. According to Porter and Baker (1992), multi-innervated fibres, in the c-shaped peripheral layer, are the first to develop. Monkey was their animal of choice but similar findings have also been reported in other mammals (Lennerstrand,1982). Gamble and co-workers (1978) found that in the human embryo the nerves to the EOM seem to reach and enter their target muscles at an early stage (50 mm). Neuromuscular contacts on the other hand become increasingly numerous with advancing age and axons seem reluctant to terminate on myofilament-poor muscle cells. The neural influence on muscle fibre proliferation during these stages , however, remains to be established. In the

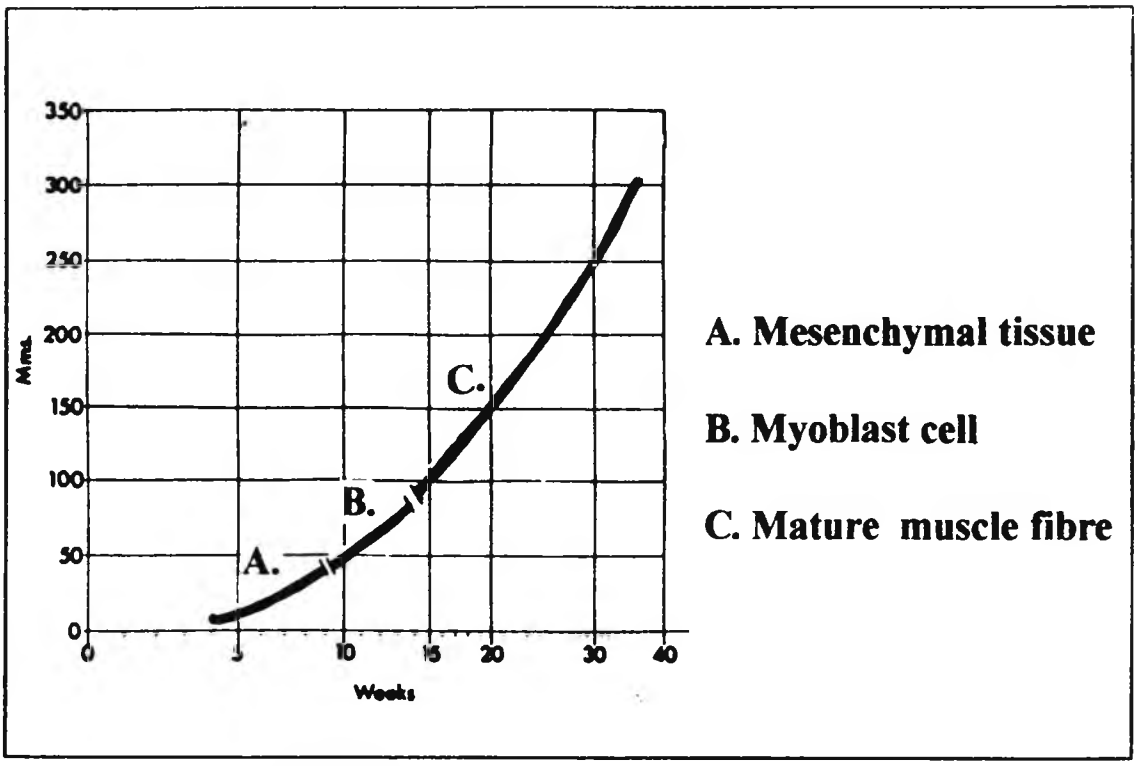


Figure 1.2 Summary of EOM development in relation to crown-rump length (mms) and age (Weeks). Design of curve is based on data from Sevel (1981).

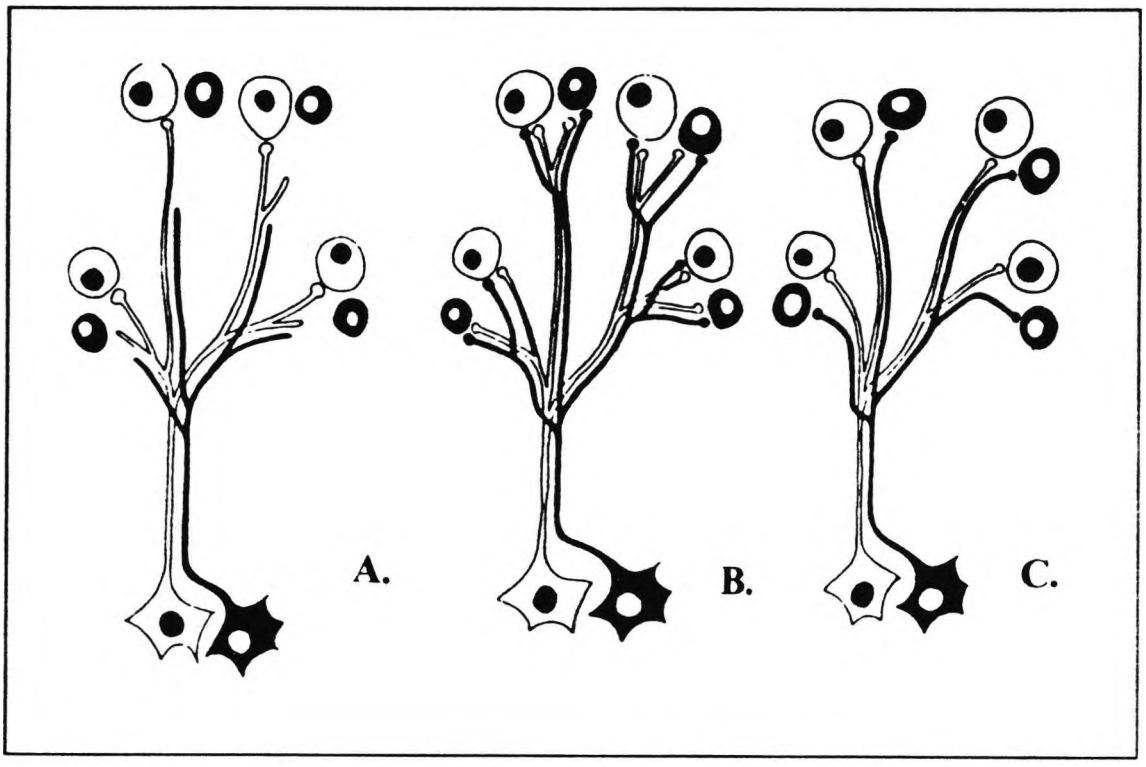


Figure 1.3 Diagrams to illustrate suggested sequence of motor axon development from polyneural (A) to mononeural (C) innervation by gradual retraction (B). (From H.J. Gamble et al. Electron microscope observations on human fetal striated muscle. Journal of Anatomy 126, 567-589. 1978)

same study Gamble observed muscle cells innervated by more than one axon. Based on this observation he postulated that polyneural innervation of muscle fibres is an early, temporary, phenomenon, which later changes into a mononeural innervation by gradual retraction (Fig. 1.3).

The nature of the multiply innervated muscle fibre in the EOM is not fully explored and the suggested presence of polyneural innervation in adult mammals is still controversial (Mukuno,1968 ; Bach-y-Rita and Lennerstrand, 1974). Most intramuscular axons seem to remain unmyelinated until the human embryo reaches about the 175 mm stage (Gamble et al, 1978). By this stage the nerve endings have become specialised and various receptors can be found. In some subhuman species the development of receptors is not completed before several weeks into life (Zelena and Soukup,1977). The state of maturity these receptors have reached at the time of birth, in humans subjects, is still unknown. Furthermore, the mechanoreceptors in the human EOM seem to have certain features which do not compare with the morphology of conventional somatic receptors. Their developmental role is still unclear.

1.1.2 Ocular Motor Nuclei and Nerves

The three ocular motor nuclei are distributed along the length of the brainstem and their position in relation to the midline varies. Their locations, in addition to the trochlear nerve's dorsal exit out of the brainstem, give rise to large variations in length of both fascicles and nerves.

The oculomotor nerve is the largest and most complex of the three. It innervates all the extraocular muscles apart from the lateral rectus and superior oblique. The superior rectus and levator palpebrae are both innervated by the superior branch of this nerve while the medial rectus, inferior rectus and inferior oblique are innervated by the inferior branch. The oculomotor nerve not only contains

somatic motor fibres but also parasympathetic fibres which synapse in the ciliary ganglion and subsequently innervate the intraocular muscles. The parasympathetic division follows the inferior branch and joins the trunk to the inferior oblique muscle for some distance. It departs from this branch of the oculomotor nerve and runs alongside the sympathetic root, composed of sympathetic postganglionic fibres that originate in the superior cervical ganglion. Along with sensory fibres of the nasociliary nerve these groups of fibres reach the ciliary ganglion. From this arrangement it follows that with the exception of a small portion of sympathetic fibres (potentially vasomotor) , the far distal end of all branches of the oculomotor nerve are virtually free of autonomic nerve fibres. In addition to these, fibres from the ophthalmic nerve have been found to accompany the orbital portion of all three ocular motor nerves. This is assumed to be true for a variety of mammals (Porter and Donaldson,1991), including man (Manni and Bortolami,1981).

The trochlear nerve is the only cranial nerve which leaves the brainstem dorsally. The two nuclei lie dorsal and medial to the MLF in the grey matter at the floor of the cerebral aqueduct. This location is only a short distance from the dorsal aspect of the brainstem. The fascicle's route is made slightly longer by the turn it makes around the cerebral aqueduct before decussating. Due to this arrangement the axons emerging from the dorsal aspect of the brainstem innervate the contralateral superior oblique muscle. The trochlear nerve is the smallest of the three ocular motor nerves, seldom containing more than 2500 axons (Torre ,1953).

In contrast to the trochlear nerve the fascicle of the abducens nerve passes by several neural structures. From its origin just beneath the floor of the fourth ventricle the fascicle runs ventrally through the pons passing the motor nucleus

and the corresponding fascicle of the facial nerve, the motor nucleus and spinal tract of the trigeminal nerve, the superior olivary nucleus, the central tegmental tract and the corticospinal tract. After passing through the annulus of Zinn it turns laterally to innervate the lateral rectus muscle. Warwick (1955) found that there is disagreement between the total number of cells present in the abducens nucleus and the number of motor axons innervating the lateral rectus muscle. These findings have been confirmed by others and have led to the conclusion that there are two primary cell populations (Büttner-Ennever and Akert,1981). One being the motor neurons producing abduction of the eye while the other gives rise to axons which join the MLF and subsequently connect the ipsilateral abducens nucleus with the contralateral medial rectus sub nucleus. (Büttner & Büttner-Ennever, 1988) .

1.1.3 Neuromuscular Junctions

There are two types of motor nerve endings in the human EOM and both of these neuromuscular junctions are of a cholinergic nature (Cheng ,1963). The most numerous of the two is the classical motor endplate where a large myelinated axon terminates on one single twitch-muscle fibre . The term «end plate» is used to describe the accumulation of mitochondria and sole plate nuclei in the sub synaptic region of these muscle fibres. The postsynaptic surface is uneven due to numerous palisade like foldings. This region accommodates acetyl cholinesterase (AChE) which is the enzyme responsible for splitting the achetylcholine complex after its interaction with the receptors on the post synaptic surface. The majority of vertebrate neuromuscular junctions have morphological features consistent with this description. The second type , is called a grape ending due to its alleged resemblance to a bunch of grapes. These endings are present on multiply innervated muscle fibres. Such fibres are only found in a few exclusive places in mammals (Fernand and Hess ,1969) but

constitute a significant portion of the fibre population in nonmammalia (Salpeter, 1987). The post synaptic junctional folds are modest on this type of neuromuscular junction and conventional endplate features such as accumulation of mitochondria and sole plate nuclei are not present. Instead of one single neuromuscular junction located in the middle third region of the muscle, this type of nerve ending has small terminal boutons distributed along the whole length of the muscle fibre. The axon leading to this so called grape ending has a diameter in the region of 1-3μm (Terävänen, 1968). When histochemical methods are applied, cholinesterase activity can be demonstrated (Dietert, 1965 ; Cheng ,1963). So, despite a clear departure from conventional morphology, the transmitter substance released at the pre synaptic junction of this synapse is cholinergic in nature. This can also be demonstrated clinically when butolinum toxin is injected into the EOM. The activity of the total fibre population in the muscle is then affected (Lee 1994 personal communication). The physiological significance of this rather peculiar nerve ending is, however, not clear.

1.1.4 Morphology

Extraocular muscles are composed of cross striated muscle fibres and should in principle have the same morphological and physiological features as other somatic musculature. It has, however, proved difficult to make direct correlations between the two. From the earliest studies the description of extraocular muscle fibres failed to conform to the classification scheme of conventional somatic muscle fibres.

Thulin (1914) described two different types of muscle fibres in the EOM of monkey and man based on differences in myofibrillar content and the presence of a rather special nerve terminal (Later found to be the grape ending described in section 1.1.3). Some years later, Kato (1938) divided these fibres into two major groups based on their size and topographical location within the muscle. The

smallest fibres were found aggregated in a semicircular layer occupying the outside (orbital) surface of the muscle surrounding a central region of larger fibres. Such disposition of fibres was later confirmed by others (Locket,1968), although large variations between species do exist (Chiarandini & Davidowitz, 1979). Fibres in the different layers were found to differ not only morphologically but also in innervation (Terävainen, 1968) and blood supply (Woodlief, 1980). Both fibre types did , however, bear structural resemblance to corresponding muscle fibres in somatic muscles of lower vertebrates. This is why they are now referred to as Fibrillenstruktur and Felderstruktur which is the terminology Kruger (1949) introduced to describe twitch and tonic muscle fibres respectively.

When stimulated, all twitch fibres propagate action potentials with a following rapid synchronised contraction. Most mammalian somatic musculature is composed of such fibres. Slow contracting fibres not capable of propagating an action potential are called tonic fibres. Although tonic fibres are primarily found in nonmamallian somatic muscles, they have been found in the tensor tympani, the stapedius and EOM of various mammals (Fernand and Hess,1969). A common physiological function for these unique muscle fibres has, however, not been identified.

Fibrillenstruktur fibre

The name Fibrillenstruktur refers to the characteristic light microscopic features of this fibre. The abundant sarcoplasmic reticulum surrounding each and every myofibril is responsible for the even punctate appearance seen in transverse section. The Z line appears straight when examined in longitudinal sections although the intermyofibral distance gives the Z line a fragmented appearance when higher magnification is applied (Fig. 1.4).

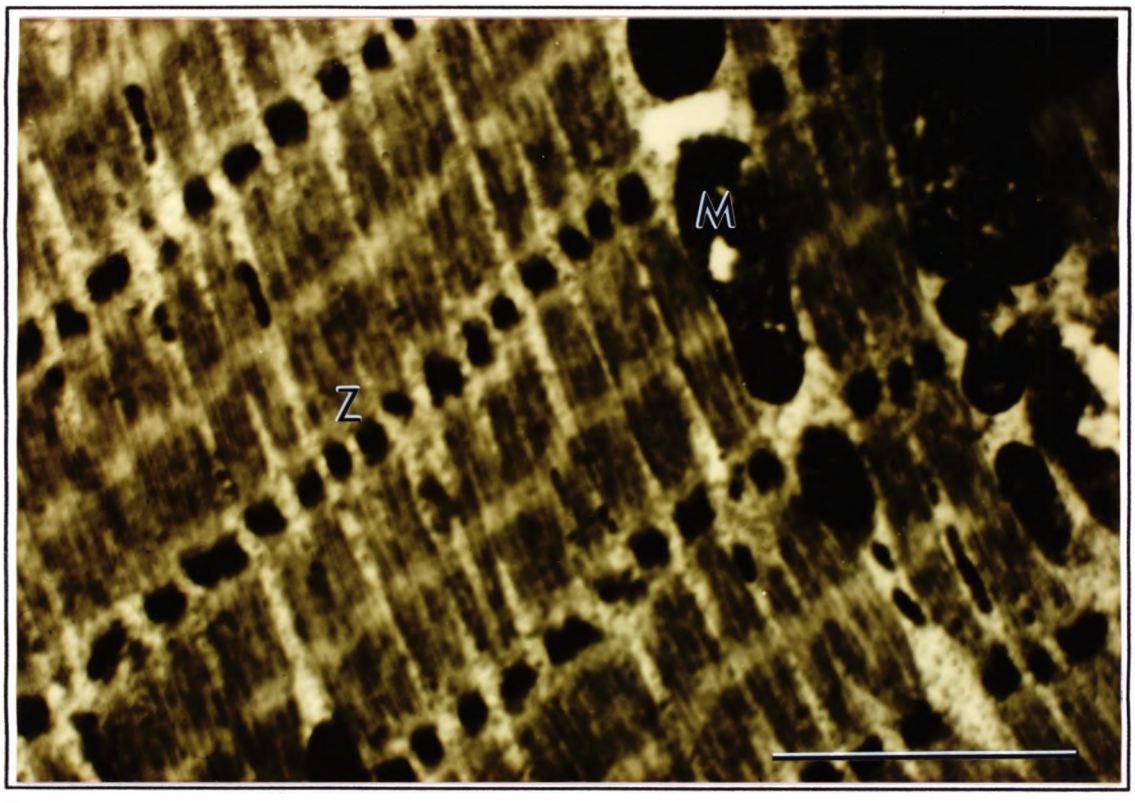


Fig 1.4 Electron micrograph of a Longitudinal section of a Fibrillenstruktur fibre of an 30 year old male. The micrograph illustrates some of the features of this muscle fibre. Separation of adjacent myofibrils by the sarcoplasmic reticulum is distinct, specially at the level of the I band located on either side of the Z line (Z). Accumulations of mitochondria (M) occur with regular intervals. The outline of the A band is visible between successive sarcomeres. Marker indicates 2μm.

Unlike their somatic counterparts the fast twitch fibres in the EOM have a high concentration of mitochondria. Although large variations do occur, these organelles tend to be pronounced. Under high magnification one can see that the sizes of the mitochondria can exceed that of the myofibrils. (Fig.1.8). Comparison between the Felder and Fibrillenstruktur fibres reveals a small diameter of the myofibrils in the latter fibre (Fig. 1.8). The diameters of the Fibrillenstruktur fibres tend to be large and fibres with diameters exceeding 60μm are not uncommon. These fibres are innervated by correspondingly large myelinated axons terminating in a classical motor endplate (Fig. 1.5 and 1.6).

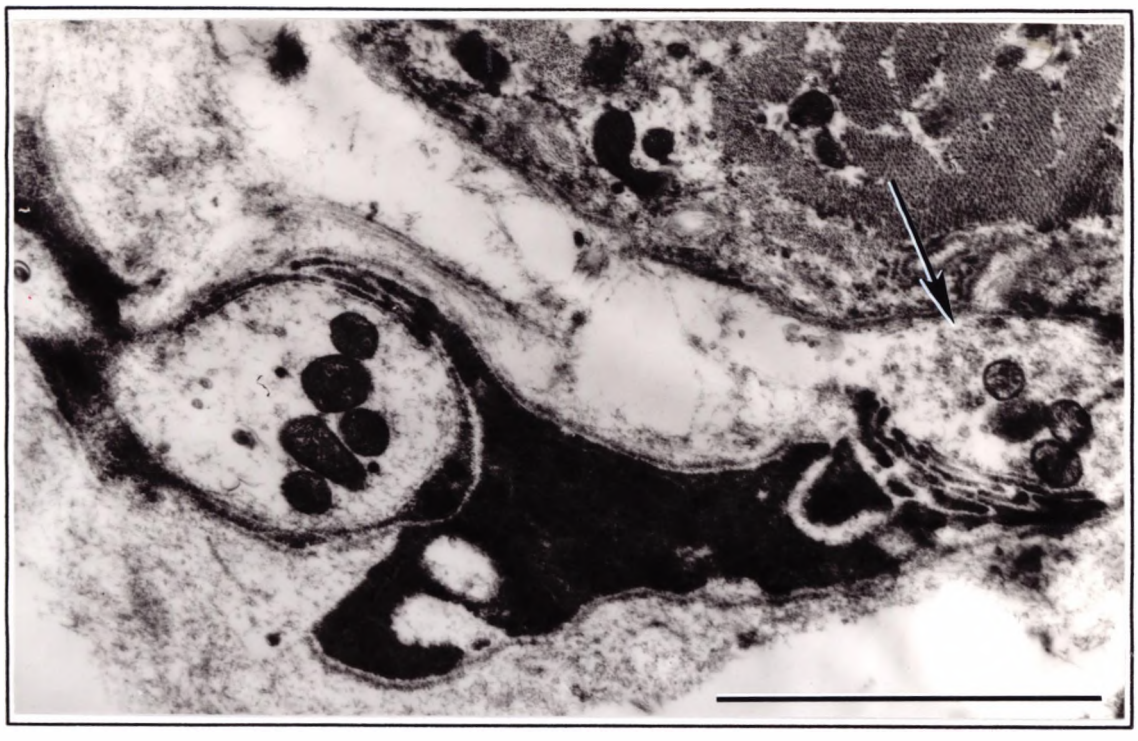


Figure 1.5 Electron micrograph of a Fibrillenstruktur fibre in transverse section showing small terminal boutons, one of them indenting the sacolemma (arrow). Sample obtained from a 30 year old male and stained with toluidine blue. Marker indicates 10μm.

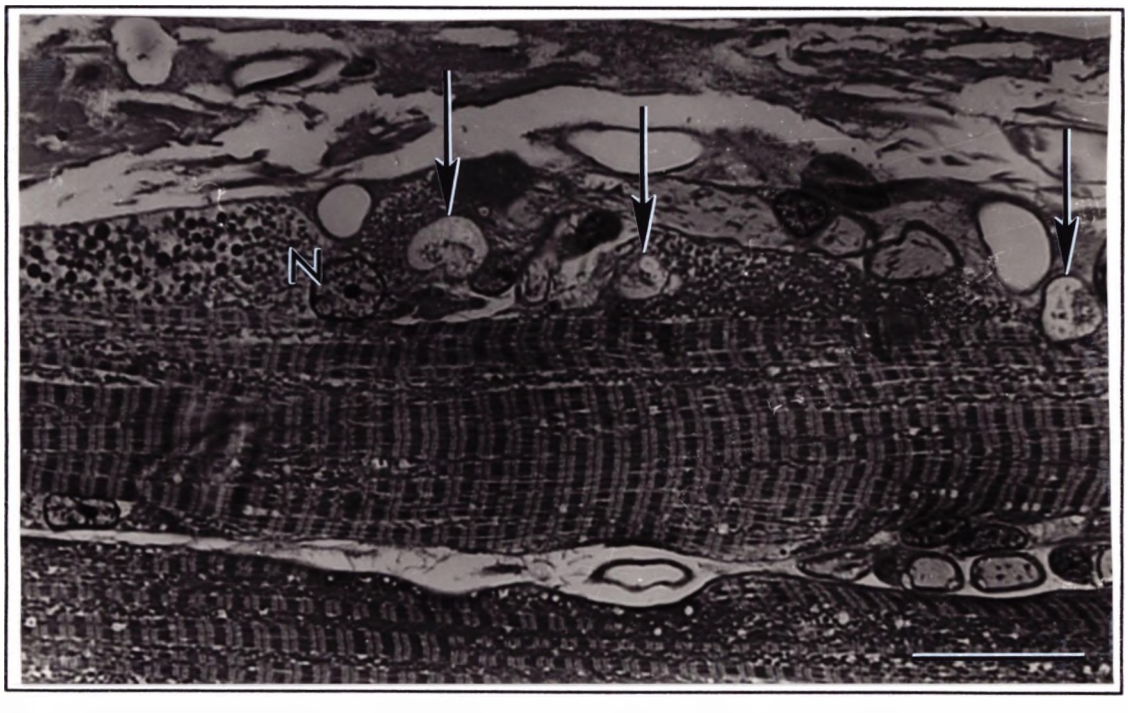


Figure 1.6 Light micrograph showing a longitudinal section through the neuromuscular junction of a Fibrillenstruktur fibre exposing the Sole-plate nuclei (N) and terminal boutons (arrows). Sample obtained from a 30 year old male and stained with toluidine blue. Marker indicates 20μm.

Felderstruktur fibres

The Felderstruktur fibres tend to be smaller in size and have fewer and smaller mitochondria than the Fibrillenstruktur fibres. The sarcoplasmic reticulum with the corresponding T- tube system is scarce, suggesting a limited release and uptake of calcium. This corresponds well with the physiological properties of the fibre. In transverse sections the lack of sarcoplasmic reticulum gives little separation of individual myofibrils. The high concentration of contractile material per square unit area gives a high absorption of stain and contributes to the dense appearance of this fibre in both light and electron microscopy (Fig. 1.7 and 1.8).

In longitudinal sections the Z line of this fibre appears thicker and more wavy than the Fibrillenstruktur fibre. The M line tends to be absent. In subhuman species the M line on the Felderstruktur fibre have been found to be area dependent, which has raised the question as to whether subdivisions of these fibres really exist (Davidowitz et al, 1981). On the other hand, results from a few electrophysiological studies on cat EOM suggests that some multiply innervated muscle fibres are capable of twitch contraction (section 1.1.5) . Such findings have not been confirmed in humans.

Criteria for muscle classification have usually been based on microscopic appearance of various structures such as the SR, T tubules, size, distribution and number of mitochondria. The variation in fibre morphology which exists between individuals, different muscles and species has resulted in complex subdivision of the two classical fibre types. As many as 6 fibres with different ultrastructural profiles have been reported (Spencer and Porter, 1988; Alvarado and Van Horn, 1974). In human muscles where the fine structure has been less

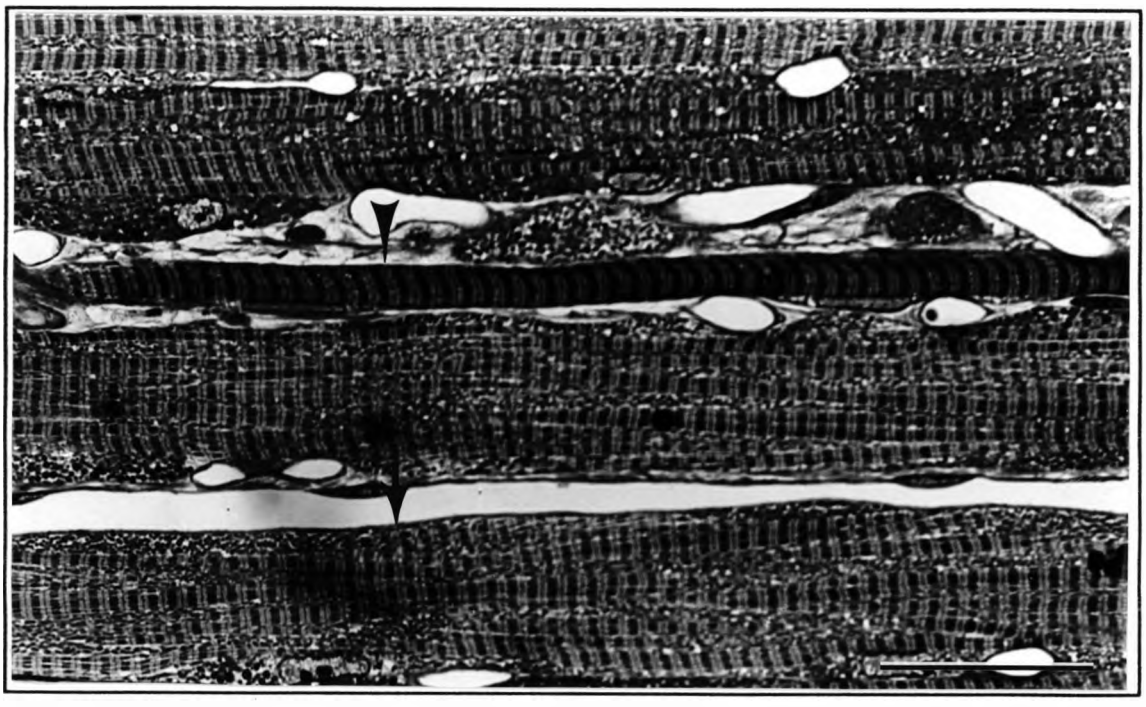


Fig 1.7 Light micrograph showing both Fibrillenstruktur fibre (arrow) and Felderstruktur fibre (arrowhead) in longitudinal section. Sample obtained from a 30 year old male and stained with toluidine blue. Marker indicates 20μm.

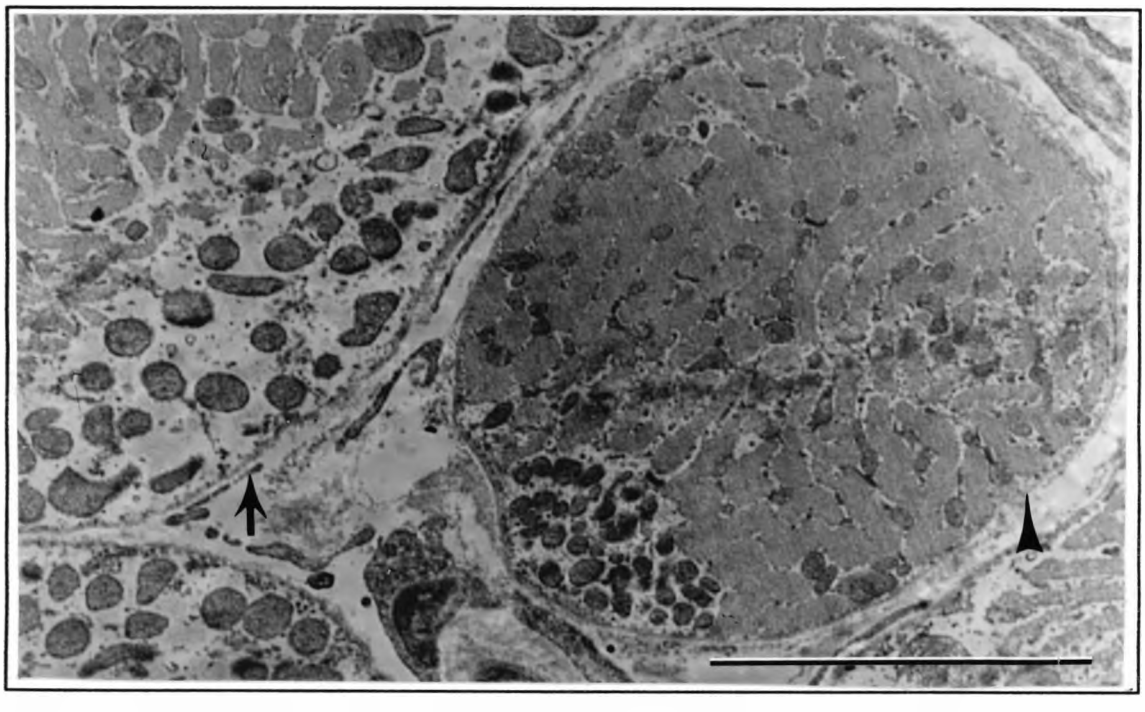


Figure 1.8 Electron micrograph of both Fibrillenstruktur (arrow) and Felderstruktur (arrowhead) in transverse section. The size of the mitochondria is notably larger in the fibrillenstruktur fibre. Some of the organelles equals or exceeds the diameter of the myofibrills. Sample obtained from a 30 year old male. Marker indicates 10μm.

exhaustively analysed , the situation seems less complicated (Dieter ,1965 ; Brant & Leeson,1966; Martinez & McNeer,1976). Here the early classifications of fibres into a fibrillar and afibrillar type still have some merit (Table 1.1)

In his study of the fine structure of the human EOM, Mukuno (1967), called the fibrillar and afibrillar fibres "F" and "S" fibres respectively. He suggested that criteria for muscle fibre classification should be based on morphologically stable organelles (i.e. T- system , SR and myofibrils). Distribution and variation of mitochondria, which are generally accepted as important indicators in skeletal muscle fibre classification, might not be equally reliable factors when applied on EOM. Although mitochondria are thought to be the principal site of oxidative reactions , they are not directly engaged in excitation- contraction coupling of the muscle fibre. Therefore the variations of form , amount and distribution of the mitochondria do not indicate directly the differences of the basic contractile

Fibre	Large Diameter Fibres	Small Diameter Fibres
Names	Twitch, Fast, Phasic, "Fibrillenstructur"	Slow, Tonic, "Feldenstructur"
Anatomy	Located mostly in central core of muscle Large diameter Nuclei peripheral Myofibrils even in size and distribution within fibre Sarcoplasm abundant between myofibrils	Located mostly in periphery of muscle Small diameter Some central nuclei Myofibrils uneven and ill-defined Little sarcoplasm amongst myofibrils
Innervation	Efferent. Single motor end plates Afferent. Simple spirals	Long diffuse motor endings Muscle spindles
Physiology	Rapid twitch contraction, "all or none" response to stimulus	Slow sustained graded contracture

Table 1.1 Table illustrating the difference between fast and slow muscle fibres (From N.A. Locket. The dual nature of human extra-ocular muscle. British Orthoptic Journal 25, 2,1968)

elements (twitch and slow contraction). Mukuno's reluctance to use mitochondria as a main criterion in classification of human EOM fibres has been justified in more recent studies. McNeer and Spencer (1981) studied muscles obtained from strabismic patients and found that mitochondrial distribution can be affected by overaction of the muscle. The fundamental alterations in strabismic patients appear to occur in the global layer, the slow fibre system and it consists of dense central mitochondrial accumulation in these fibres. These findings suggest that function may, to some extent, dictate morphology and not the other way around. Combinations of histological and histochemical methods have been used on human EOM by several workers (Ringel et al 1978; Hoogenraad et al, 1979 and Nunomura et al, 1984). Although the introduction of histochemistry helped to narrow the gap between the morphology and biochemistry of tissues in limb muscle, it seems to be of less value when applied on the EOM.

Ringel and co-workers (1978) described three different fibre types by using the same histochemical battery as used on limb muscle. The histochemical features of these fibres were found to correspond with the granularity which occurs in transverse sections when trichrome stain is applied. The granular, coarse and fine fibres owe their characteristic microscopic appearance to mitochondrial distribution and myofibrillar architecture (McNeer and Spencer, 1981). The coarse fibre was thought to be multiply innervated (equivalent to the Felderstruktur fibre) while the granular and fine corresponds to fast and slow fibres respectively. Most fibres in the orbital surface layer are coarse fibres. These fibres also constitute about a third of the fibres in the global layer. The remaining fibres are largely of the granular type with only a small portion of fine fibres . These findings suggest that the Felderstruktur fibres are dominant in EOM. With the exception of a solitary observation (Sadeh and Stern, 1984), there is no support of such a fibre distribution when other methods are applied.

Later histochemical and ultrastructural studies showed that only small fine fibres were multiply innervated (Porter et al, 1995). Histochemical and biochemical properties of the EOM are not investigated in the present study and will therefore not be discussed any further.

1.1.5 Physiological Properties

Hess (1961) studied the ultrastructure of the EOM in guinea pig and found two distinct morphological fibre types. In co-operation with Pilar, he extended this study and tried to correlate morphology with function, this time in cat EOM (Hess & Pilar, 1963). They recorded two types of contractile activity in these muscles. The fast activity was associated with the twitch fibres while the slow movements were linked to tonic fibres. These findings confirmed that a slow and fast system is also present in EOM and is not only a feature of somatic muscles in lower vertebrates (Kuffler and Williams, 1953).

Hess and Pilar's study initiated numerous theories on the function of EOM in oculomotor control. The absence of a slow system in the levator palpebrae, which also receives innervation from the oculomotor nerve, was taken as evidence for a selective unique organisation of the ocular rotary muscles (Dieter, 1965). Various clinical conditions seemed to fit the favoured hypothesis of two separate motor systems. For instance, the reduction of convergence seen in patients suffering from internuclear ophthalmoplegia was argued to be caused by a lesion affecting the innervation to the Felderstruktur fibre. The concept of a dual nature of the human EOM was therefore thought to have extensive implications for orthoptists who are dealing with disturbances of the EOM (Locket, 1968). But, despite diligent search, the anatomical evidence for two separate motor systems was not found. Papers which followed argued against the dual theory, claiming that there is insufficient evidence to ascribe specific functions to specific muscle

fibres (Breinin ,1971). Scott and Collins (1973) forwarded this view most clearly in their paper on electromyographic recordings from human EOM. They found that motor units which altered activity during one type of eye movement, for example saccades, did so during fixation, following, and vergence movements as well. On this basis they claimed that motor units change activity based on the muscles` need for more or less action, independent of the type of eye movement being performed. These findings quite clearly oppose the theory of division of labour. On the other hand, even though various populations of muscle fibres cannot be found to serve separate eye movements, muscle fibres might still be biased in their contribution to a specific movement. An eye movement can for instance be extremely rapid, up to 600 degrees of arc per second (Porter and Baker, 1992). The Felderstruktur fibre's contribution in such a movement must arguably be very modest. Hess & Pilar clearly demonstrated that no spike action potentials could be elicited by stimulation of the nerve to the slow fibre system. However, due to potential sources of error in the applied method, they could not state with any certainty that these fibres usually do not produce propagated action potentials. The lack of conclusive results inspired further electrophysiological studies and three years later a third fibre was reported, a multiply innervated fibre with action potentials (Bach-y-Rita and Ito, 1966). Attempts to confirm these findings were unsuccessful (Pilar,1967) until Lennerstrand published his study on the electrical properties of the cat EOM (Lennerstrand, 1975) . These observations gave a better consistency between the number of fibres found morphologically and physiologically. The three different fibres were classified as *Twitch, single innervated fibres (SI), Tonic multiply innervated fibres without action potentials (MINC)* and finally *Multiply innervated fibres with action potentials (MIC).*

The concept of a multiply innervated twitch fibre is still controversial and (as previously stated) no such fibres have been identified in man. However, sophisticated physiological studies on human muscles are in general impractical. Knowledge of the physiological properties of human EOM is therefore sketchy. Classification of the various fibres is based on morphological characteristics and their physiological properties have been assumed by correlation with similar fibres in closely related mammals.

The EOM are clearly unique when compared with conventional skeletal muscles, not only due to their structural and functional characteristics but also in the kind of work they perform. In skeletal muscle the tension which develops during a contraction will vary with the effect of gravity and posture of the body. Musculature of the extremities are frequently burdened with external loads, hence the effect of stimulating a fixed number of motor units cannot be predicted. A stimulation initially giving rise to an isotonic contraction can, when repeated, result in an isometric contraction if the load has changed. The situation for the extrinsic muscles of the eyes is quite different. The eye's centre of gravity lies close to its centre of rotation. This relationship will nearly eliminate the effect of gravity. Since no additional weight can be applied on the eye which is in itself rather light, the load on the EOM remains fairly constant. The modest tension required to move the eyes from one stationary position to another reduces the amount of isometric contraction these muscles have to undergo. These distinct working conditions have consequences for both the recruitment of motor units and the necessity to monitor the muscle tone. These issues are fundamental for the understanding of oculomotor control. Even so , the literature presents conflicting views , which suggests that further studies on the motor and sensory innervation of the EOM are still needed.

1.2. MOTOR UNITS

A motor unit consists of a motor neurone, its axon and all the muscle fibres it stimulates . The term "motor unit" was introduced by Sherrington (1925) and refers to the restricted activity one component of the unit can have without a corresponding activity in the others. The axon of the motor neurone will divide several times once inside the muscle and each branch terminates on one muscle fibre alone. All the muscle fibres within one unit have similar morphological features and share the same physiological properties. This seems to be true for both EOM and somatic musculature in general .When stimulated , not only will the fibres contract simultaneously but also with the same velocity, force and duration. The total muscle activity is determined by the number of motor neurones involved at the given time and any increment is due to excitation of an additional motor unit. The alteration in muscle force which follows each new recruitment is determined by the number of muscle fibres and their contractile properties (i.e. size and nature of unit). This makes the motor unit the smallest functional unit in the muscle and it is therefore not surprising to find variations in the size of this unit throughout the body. Small units are usually found in muscles controlling minute movements. Fine muscles of the hand along with facial muscles and muscles in the larynx belong to this group. These muscles, however, do not match the motor unit in the extrinsic muscles of the eye which is the smallest in the body (Goldschmidt, 1969).

Muscle fibres belonging to the same unit are not necessarily located in the same fascicle and a broad spectrum of fibres can be found within a restricted area of the muscle. In skeletal musculature there seems to be a correlation between the size of the muscle fibres and the motor unit. The small units contain muscle fibres of lesser diameter innervated by small diameter axons . Recruiting such a unit will add little extra force to the muscle contraction, suggesting that these

units are valuable in finer adjustments of muscle force. Recruitment of large units will in contrast add considerable force and velocity to the contraction. The system of recruitment of motor units according to size (the size principle) suggests that the initial recruitment of small units gives precise movements while large units will be involved if rapid forceful movements are required.

Although the richness of efferent fibres have been commented upon by many workers, there is uncertainty as to the size range of the motor units in human EOM. Whether the morphological basis for a size principle is applicable in these muscles is therefore a matter of debate. One could argue that the necessity of a wide span of motor units of various sizes is less in the oculomotor system. In contrast to the somatic system, the division of labour in the oculomotor system seems to be related to the nature of the muscle fibres within the unit , rather than to the size of the unit itself. Even though Scott and Collins (1973) provided evidence against the speculation that various types of eye movements are served by separate populations of muscle fibres, the existence of two such morphologically distinct fibres must clearly have some functional implications. The nature of the motor unit serving the slow fibre system has been subject to most speculation. Fibres within this unit have their counterpart in amphibian muscle, where they are supposed to produce the slow and long lasting contractions that are used in some of these animals' motor acts (Lännergren, 1974). Scott (1971) suggested a similar function for the slow fibres in EOM, i.e. to supply the strong tonic activity in eye fixation.

The modest size of the motor units in the oculomotor system is undoubtedly of great significance, but cannot be the only contributing factor to the fine gradations in motility of the eye. A pure recruitment of new motor units would create a step like increment of muscle force unless the discharge frequency in the

last recruited motor unit could be adjusted. The two principal ways of adjusting a muscle contraction are therefore by altering the number of motor units involved or by changing the discharge frequency. A combination of the two methods is applied in the majority of cases in order to avoid jerky movements.

The muscle force is not determined solely by the these two factors . The length of the sarcomere at the time of stimulation also influences the muscle force. In a stretched muscle the cross bridges between actin and myosin filaments will be few. Since interaction between actin and myosin is essential for muscle force it is evident that a stretched muscle is not the ideal starting point for muscle activity which (initially) is always isometric in nature. A similar reduction in muscle force will occur in a muscle with short sarcomeres. In a contracted state the actin filaments will approach the mid region of the myosin filaments, which is free of binding sites. If the sarcomere is in an even more foreshortened position the actin filaments will have reached the opposite region of the myosin filament, where myosin heads with opposite polarity are located. Maximum muscle force is therefore developed when the muscle is positioned in such a way that the sarcomere length is somewhere between maximum and minimum length. This relationship is demonstrated in figure 1.9.

If the filaments slide past each other with a high velocity, there will always be cross bridges in the process of breaking up or finding new binding sites. Since single myosin heads disconnected from a binding site cannot contribute to the muscle force it follows that force is lost with increasing contraction velocity. There is therefore a clear relationship between speed of contraction and load on the muscle. In contrast to skeletal muscles, the load on the EOM is modest and more important, fairly constant. However, the arrangement of the muscles into

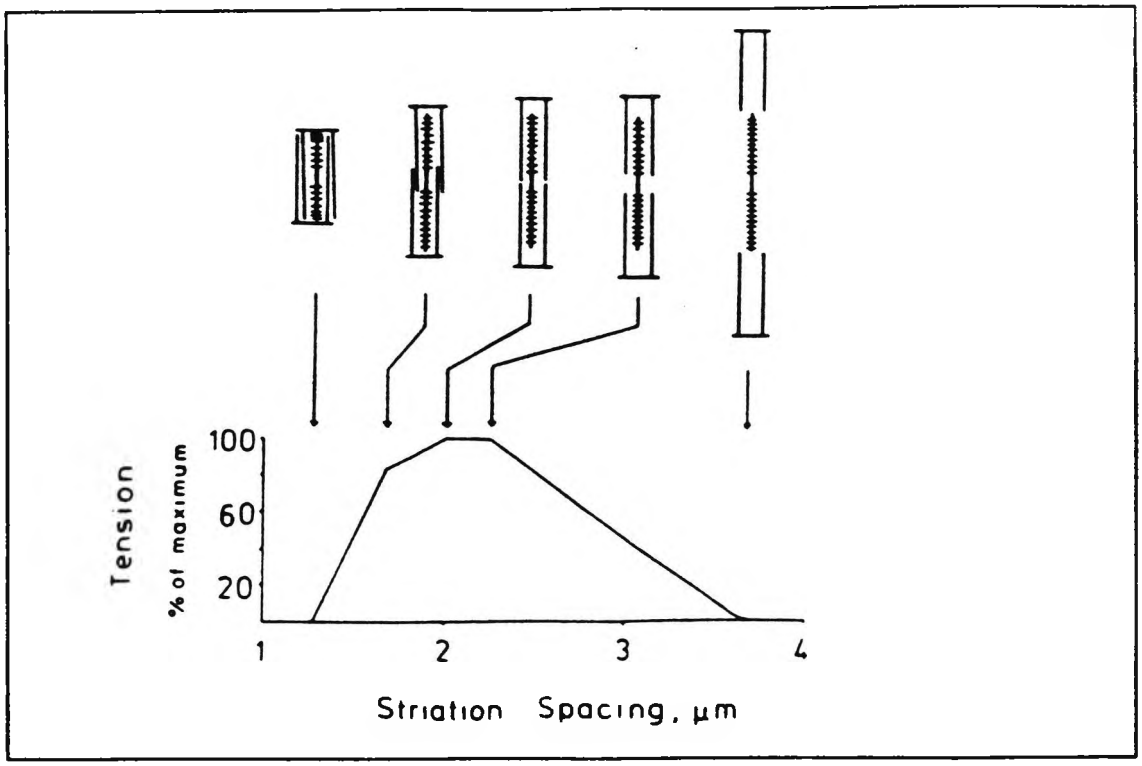


Figure 1.9 Diagram showing the relationship between muscle force and filament overlap. (From H.M. Eggers. Functional anatomy of the extra ocular muscles. In Ocular Anatomy, Embryology and Teratology. Editor Jacobiec. Harper & Row, 1982)

three antagonistic pairs ensures that opposing forces are always present. Tension will also be created by the combined non- muscular tissues (e.g., Tenon's capsule, optic nerve, suspensory ligaments etc.) The tension created by these tissue elements can be found by simply rotating the globe to various fixed lateral deviations. The resistance to displace the globe from its position of rest increases with the degree of deviations. From this relationship follows a tendency to restore the eye to primary position.

The motor unit is used as an indicator of the physiological properties of the respective muscles . However, by simple light microscopic observation, it is virtually impossible to distinguish between axons relating to different functions. Quantitative studies of efferent nerve fibres based on a single histological cross section will inevitably include afferent nerve fibres as well. This is suggested

when histograms of the nerve fibre spectra are produced, revealing bimodal and even trimodal distributions (Feinstein, 1954). Adjustment of the obtained data is sometimes attempted, based on the following assumptions:

a) All efferent nerve fibres innervating extrafusal muscle fibres are large.

b) Small efferent nerve fibres go to intrafusal fibres.

c) Large sensory nerves are concerned with proprioceptive sensation while

d) Small unmyelinated/myelinated axons subserve pain and autonomic functions.

Regarding the EOM, these assumptions are invalid. The majority of the efferent nerves are arguably of the large diameter type, but they are not the only motor nerves innervating these muscles. Felderstruktur fibres which are innervated by small nerve fibres constitute about a fourth of the muscle fibre population. Furthermore even though sensory receptors do exist in EOM, their nature, amount and distribution have not yet been investigated fully.

The initial attention which has been drawn to the motor unit in human EOM is not recent (Tergast, 1873). In most studies the method of choice has been estimates from low resolution micrographs. Data from various studies are summarised in the Table 1.2.

The efferent fibres in the ocular motor nerves span a large range from 15-20 μm down to less than 1μm. The method of observation applied must therefore include high magnification. Nerve fibre counts from micrographs with magnifications below 100 (Kato,1938 and Goldschmidt,1969) cannot possibly include the small fibres in the far end of the spectrum. At this level of magnification even the high concentration of blood vessels represents a potential element of error. Comparison of the results of the various publications is

hindered by failure to state the position from where sections were taken. Further study on the motor unit, applying methods of high accuracy , is therefore needed.

	TERGAST (1873)	TORRE (1953)	BORS (1926)	GOLDSCHMIDT (1969)
MEDIAL RECTUS	3282:5580 (1:1.7)		4552:21950 (1:4.8)	10733:41575 (1:3.9)
INFERIOR RECTUS	5175:10351 (1:2.0)		3303:20809 (1:6.3)	
LATERAL RECTUS	3625:11965 (1:3.3)	5000:24400 (1:4.9) 3300:19760 (1:6)	4698:27214 (1:5.8)	4800:31639 (1:6.6)
SUPERIOR RECTUS			(1:4.3)	
SUPERIOR OBLIQUE		2440:13140 (1:5.3) 2100:14220 (1:6.8)	2858:14866 (1:5.2)	
INFERIOR OBLIQUE			2508:17557 (1:7.0)	

Table 1.2 The table summarises the results from various studies on the motor unit in human extraocular muscles. The figures represents muscle and nerve fibres reported and expressed as a ratio (motor unit in parentheses) .

1.3. SENSORY RECEPTORS

The term "proprioceptor" embraces all sensory end organs capable of providing the central nervous system with information about body movements. Functionally they are classified as low threshold mechanoreceptors and their signals are conveyed in thick myelinated axons.

1.3.1 Muscle Spindles in Somatic Muscles

The muscle spindle, being an example of a mechanoreceptor, can be found in large numbers embedded in connective tissue between bundles of muscle fibres. Their concentration, distribution and size vary with the morphology and physiological properties of the muscle in which they lie. The highest concentration of spindles is usually found in muscles taking part in delicate movements, which suggests that these muscles need a more extensive sensory input (Barker, 1974). In the literature the EOM are frequently used as an example of such muscles. But even though there is a generous complement of spindles in the EOM of many species, their importance in ocular motor control is still controversial. In humans, the problem is not only linked to the rather peculiar morphology of this receptor but also to the value of the potential sensory information it could provide. It is generally accepted that spindles in somatic musculature participate in stretch reflexes. Displacement of body weight, which follows unintended sudden movements, would lead to loss of balance without the counteracting contraction initiated by the spindles. The same reflex is able to prevent undesirable changes in muscle length caused by changes in the load acting on a muscle. Furthermore, the sensory neurones from the spindle also relay impulses centrally permitting judgement of the position and movements of our extremities (Carpenter, 1988).

1.3.1.1 Morphology

The muscle spindle consists of several short, fine muscle fibres of unique form enclosed by a capsule and attached by connective tissue at each pole to the main muscle mass. The capsule, a continuation of the perinurium, is closed at each pole and fluid-filled and its diameter is expanded centrally giving the structure its spindle shape. The 1-15 spindle, or intrafusal, fibres lose much of their contractile structure towards the centre or equator of the spindle by a reduction in diameter and replacement by centrally placed nuclei. One type of fibre has a single row of nuclei, another a broader accumulation of nuclei, respectively called nuclear chain and nuclear bag fibres (Cooper et al., 1955). In human adult muscle tissue the equatorial region of the spindle is of the order of 200μm while the length varies from 1000 to 1500μm. All intrafusal fibres in somatic musculature receive a sensory innervation, the thick myelinated axons (Barker, 1974) terminating as annulospiral endings on the modified region of the fibres. A secondary sensory innervation is also present distributed predominately to nuclear chain fibres, and both receive efferent axons terminating on their polar regions. The motor nerves serving intrafusal fibres are referred to as gamma efferent to distinguish them from the larger motor nerves ending on non-spindle or extrafusal fibres (Leksell, 1945).

1.3.2 Spindles in Extraocular Muscles

The functions attributed to proprioception in somatic muscles are not necessarily valid for EOM. For example the eye's centre of gravity corresponds well with its centre of rotation and the effect of gravity will therefore not act upon the muscles during rotations of the eye (Collins, 1971). Furthermore, the EOM loading remains fairly constant during normal eye movements. These factors probably explains the lack of a stretch reflex in the oculomotor system (Breinin, 1957; Keller and Robinson,1971). One could further argue that there is no need for spindles as a provider of eye position sense since this function is already

fulfilled by sophisticated exteroceptors in the retina (photoreceptors). Helmholtz (1867) was the first to forward this view, and claimed that visual information from the retina along with knowledge of the voluntary effort renders proprioception unnecessary . Even without the visual input it is clear that the oculomotor system departs from the conventional somatic system in more than one way.

These fundamental differences have led to speculations as to the true function of spindles in the EOM. The presence of spindles in some species but not in others makes the potential role of this receptor even more obscure, and these early deficits were probably responsible for the oversight of initial reports on muscle spindles in human EOM. Buzzard (1908) claimed that spindles were present in human EOM and he was the first to convincingly support his observation by producing fairly detailed micrographs in his report. But it was not until Cooper and Daniel (1949) published their work on spindle morphology that these receptors were fully recognised as true muscle spindles. In the papers which followed the human EOM spindles have been described as small and delicate , which is claimed to be the main reason for their late discovery. These and other features peculiar to spindles were described by Ruskell in 1989 and led to the contention that they are probably incapable of proprioception. This observation, made from aged muscles, is intriguing and encourages review of the role of EOM proprioception in general oculomotor control.

1.3.2.1 Morphology

The diameter of the human EOM spindle is in the region of 40- 80 μm. The widest periaxial space is usually found in long spindles which can exceed 1000 μm in length (Merrillees et al 1950). But a section through the equatorial region of even the largest spindles does not reveal much periaxial space. This rather

crowded appearance is due, not only to the spindle's modest size, but also to its content. As few as one and as many as 16 intrafusal fibres are present, a range which compare well with conventional spindles (Ruskell, 1989). However, the difference in diameter between the extrafusal and many intrafusal fibres is frequently minimal.

With a modest equatorial diameter the capsular outline becomes less spindle shaped. The continuation of the perineurium, forming the capsule, is augmented by a few thin connective tissue lamelly. Fibrous connections crossing the periaxial space and formation of an inner capsule, which is usually pronounced in the conventional spindle, are far less distinct in the EOM spindle. The content of connective tissue in some spindles is, however, plentiful but disorganised and some intrafusal fibres can even be found embedded or attached to the capsule wall.

It is not only the size of the intrafusal fibres which differs from the conventional fibres but also factors such as nuclear distribution and innervation. Nuclear chain and bag fibres are both present but the modest number of nuclei seen abreast in the latter fibre hardly justifies the name «bag fibre» (Cooper and Daniel,1949 ; Ruskell,1989). Other spindle anomalies such as displacement of the sensory region of the fibres in relation to the equator and the presence of anomalous intrafusal fibres with extrafusal morphology are features exclusive to human EOM. Although previously observed , anomalous intrafusal fibres had never been subjected to detailed study until recently (Ruskell,1989). Their presence in the spindle is intriguing because they do not compare with other intrafusal fibres and their potential physiological properties cannot be linked to any known spindle function. They have myofilamentous material along their whole length with no modified equatorial region. This suggests that they would oppose being stretched during extension of the spindle and having no connections

with afferent fibres, they probably contribute nothing to spindle responsiveness is hard to see how they can fulfil a sensory role.

The highest concentration of spindles is found in the proximal and distal end of the muscle away from the motor end plate region and as many as 71 have been reported in one muscle (Merrillees et al,1950). Analysis of muscle spindles is, however, usually based on observations of encapsulated fibres in transverse sections. Such a method of counting would cause an element of error when applied to extraocular muscles. Among the generous nerve supply to EOM there are axons which frequently share their perineural tissue with neighbouring muscle fibres. Capsules enclosing single muscle fibres were first observed associated with motor endings (Ruskell ,1983) but sheathing of muscle fibres remote from such areas has also been reported and referred to as false spindles (Ruskell,1984). It is reasonable to assume that earlier counts of muscle receptors in human EOM include these false spindles and that muscle spindles are in reality less numerous. At the time of writing, this assumption was confirmed . Lukas et al. (1994) found that the mean number of spindles did not exceed 34 in any of the six extraocular muscles. The highest concentration of spindles was found in the inferior rectus while the inferior oblique had the lowest. However, spindles were found to be at least as frequent in human EOM as in those somatic muscles regarded as possessing a high density of spindles. Lukas and his co-workers concluded that the density and regular distribution of these spindles suggest that spindles are functionally important proprioceptors in EOM.

1.3.2.2 Potential functions

Position sense

Sherrington (1918) thought that muscle spindles could give us conscious sense of eye position. But even though the human EOM is adequately equipped with

both spindles and other mechanoreceptors , there is little evidence to suggest that these mediate any substantial position sense. Skavenski et al (1972) demonstrated the presence of a feeble position sense by passively displacing the eyes of the observer in the dark. A reduction in spatial location has also been reported in patients suffering from herpes zoster ophthalmicus. In this condition the oculomotor nerves are not affected. The patient's reduced hand/eye co-ordination in the active phase of this condition has therefore been put down to interference in proprioception (Campos et al,1986). Merton (1961) claims that there is no need for spindles because there is no reason why we should not be able to judge the size of the motor volleys leaving the brain as accurately as we can judge the size of the sensory volley arriving. His view is supported by observations of patients unable to determine the direction of an eye deviated by forceps. Helmholtz (1867) held this view long before there was any detailed conception of muscle sense. He did, however, emphasise that muscle sense is unnecessary only when the motor signal is assisted by messages from the retina. It seems that if the muscle spindles in extrinsic eye muscles convey muscle sense information at all they only gives rise to a feeble position sense. This fact does not preclude the possibility of having other functions at a subconscious level.

Stretch reflex

As the EOM are not subjected to changes in load, there will be a substantially unchanging relationship between the degree of activation of the eye muscles and the resultant rotation of the globe, consequently, the need for a stretch reflex has been questioned and indeed one has not been demonstrated (Keller and Robinson, 1971; Breinin, 1957). However, Baichenco et al.(1968) pointed out that the unique features of the fibres in EOM might dampen the reflex. If the stretch reflex only invokes slow Felderstruktur fibre activity then the slow sustained changes in tone generated could easily be overlooked. The fact that

such a response has still not been reported , 25 years later, argues against this view. Failure to demonstrate a stretch reflex has led to numerous theories regarding the true function of muscle spindles. Regardless of the spindles` capability to create a stretch reflex, it could still play a vital role in the development, maintenance and stability of oculomotor control.

Development

Appropriate binocular retinal stimulation depends on a normal development of ocular movements. Information regarding developmental changes in muscle fibre content is limited but age related differences have been found (Goldschmidt, 1969 ; Kato, 1938). Ignoring the potential element of error caused by variation in fibre content along the length of the muscle, these observations suggest that in a growing muscle the force resulting from a set discharge frequency will change with time. In order to prevent a divergence between a desired eye rotation and the one actually being performed , the muscle length must somehow be constantly monitored and tuned. Muscles with different contractile properties require different amounts of innervation. Tuning of the oculomotor system cannot therefore be based on the relationship between the discharge frequency and corresponding contraction in one muscle alone. It must include all muscles involved in the rotation of the eye. This requires a rather sophisticated monitor system which by some is suggested to be based on proprioception. Surgically interrupted proprioception in developing cats has been found to give deficits in visually guided behaviour (Hain and Diamon,1983; Trotter, Fregnac and Buisseret,1987). The oculomotor system of the cat does, however, differ from man's not only in general structure but also in the lack of spindles. The value of these observations as a support for a developmental role for spindles is therefore marginal.

Oculomotor control

Compared to somatic musculature, the EOM have fairly stable working conditions. The load , however, is only one among several parameters affecting the contraction process. The absence of a variable load acting upon the EOM is therefore in itself no obvious reason to exclude the spindle as an essential element in oculomotor control. Various factors such as growth, age, degeneration, metabolic activity and pathology might alter the relationship and cause discrepancy between intended and actual eye rotation. A control system solely based on the efferent signals would represent an open loop system which would not be capable of detecting such deviations. It has been suggested that the oculomotor system is more selective and that proprioception is compared with the efferent signal to verify that the motor signals being sent are actually utilised (Matin, 1986) . The fixation instability which is present in humans suffering from congenital nystagmus resembles the oscillating movements observed by Fiorentini and Maffei (1977) in cats with the ophthalmic division of their V nerve cut. Optican and Zee (1984) suggested that an abnormality in afference may be responsible. Mitsui et al(1986) suggested proprioception to be implicated in the aetiology of strabismus.

1.3.2.3 Possible central projections

Whether or not EOM spindles provide a conscious or subconscious afferent input for the control of ocular motility, branches connecting oculomotor nerves with the trigeminal nerve have been found in various mammals, including man . Cooper and co-workers found in 1955 that afferent fibres from goat EOM travel intraorbitally to a branch of the trigeminal nerve and enter the brainstem with it. Porter and Spencer (1982) made a similar observation in cat and monkey. They believed that neurones subserving sensory innervation of the EOM travel in the ophthalmic nerve to the trigeminal ganglion where they synapse. In other studies

the first order neurone of the EOM proprioceptors has been located in the mesencephalic trigeminal nucleus (Batini and Buissert,1974: Cooper, Daniel and Whitteridge,1955) and in the cuneate nucleus in the medulla (Porter, 1986) Quantitative studies of the neural connection between the oculomotor nerves and the ophthalmic branch have raised the question as to whether this is the only route for proprioceptive information. Following intracranial ophthalmic neurectomy in monkeys, Ruskell (1983) found that the few ophthalmic nerve fibres entering one of the EOM appeared inadequate to serve the large number of receptors present in these muscles. Despite these findings most recent papers seem to favour the ophthalmic nerve as the main route (Miller, 1985). The further pathway from the trigeminal nucleus to the thalamus and cortex is unexplored in man.

1.3.3 Tendon Receptors

In somatic musculature, where the muscle spindles are believed to be fully operative, additional proprioceptive information is provided primarily by the Golgi tendon organs. Although these structures are organised in series with the muscle fibres and located further away from the contractile components of the muscle, they are now recognised not only as tension transducers but also as contributors to position sense (Brodal, 1990).

Golgi tendon organs were first found in skeletal muscle but were initially overlooked in ocular muscles (Golgi,1880). Nearly three decades later, Dogiel (1906) claimed that tendinous sensory receptors are present in the EOM of a variety of animals. More recent studies at electron microscopic level have reported diversities in the morphology of these structures which seems to be species dependent (Ruskell, 1978 and 1979:Alvarado-Mallert and Pincon-Raymond, 1979). Dogiel (1906) included human eye muscles in his survey but it took a long time before the presence of muscle tendon innervation was

confirmed. Cooper and Daniel(1949) and later Cooper,Daniel and Whitteridge (1955) claimed that such endings are present but did not describe them in detail. Their attention was directed towards what they regarded as the main contributor of position sense; the muscle spindle and spiral nerve endings. The latter structure was later found to be of efferent origin and to terminate on motor endplates (Ruskell,1983). In 1983, Mukuno reported structures similar to GTO but did not describe them. The following year, Richmond and co-workers (Richmond et al, 1984) found nerve terminals in the tendinous insertion of human eye muscles which resembled the so-called palisade ending, previously described in EOM of cat (Alvarado-Mallert and Pincon-Raymond,1979).

The only report on the fine structure of the receptors at the myotendinous junction of human EOM does not compare with previous light microscopic observation, nor with receptors described in other species (Sodi et al,1988) . There are however similarities between all these structures and their differences might be expressions of phylogenetic and/or developmental factors. This view is supported by observations in developing animals where transient neuromuscular contacts have been found. These structures might be precursors of mature Golgi tendon organs (Zelena and Soukup, 1977) and a common origin of myotendinous receptors in EOM with diversities in the mature form is a possibility (Ruskell,1979). This notion justifies the following brief review of these structures:

1.3.3.1 The Golgi tendon organ (GTO)

Golgi tendon organs are encapsulated sensory endings distributed along musculo-tendinous boundaries of most striated somatic musculature. Each receptor lies in series with a discrete number of muscle fibres, usually 3-25 fibres per tendon organ . These fibres converge sharply before they terminate in the musculo-tendinous junction at the vicinity of the proximal margin of the receptor

(Fig. 1.10). The capsule, which is 8-10 times longer than it is wide, is confluent with the perineurial epithelial sheet of the nerve fibre innervating it, forming collars at the proximal and distal ends, tightly enveloping the bundles of collagen occupying the lumen at either end. The capsular space is fluid filled and isolated from surrounding fluids by the seals formed by the collars.

In contrast to the muscle spindle, the tendon organ is supplied only by afferent nerve fibres. The relationship between the capsule and the perineurium of the primary afferent, which belongs to the group of myelinated rapid conducting sensory nerve fibres, enters the capsule of the receptor and numerous unmyelinated axon branches form spirals around discrete collagen bundles. Golgi tendon organs act as transducers in a feedback circuit that regulates muscle force in a manner similar to the spindle circuit that regulates muscle

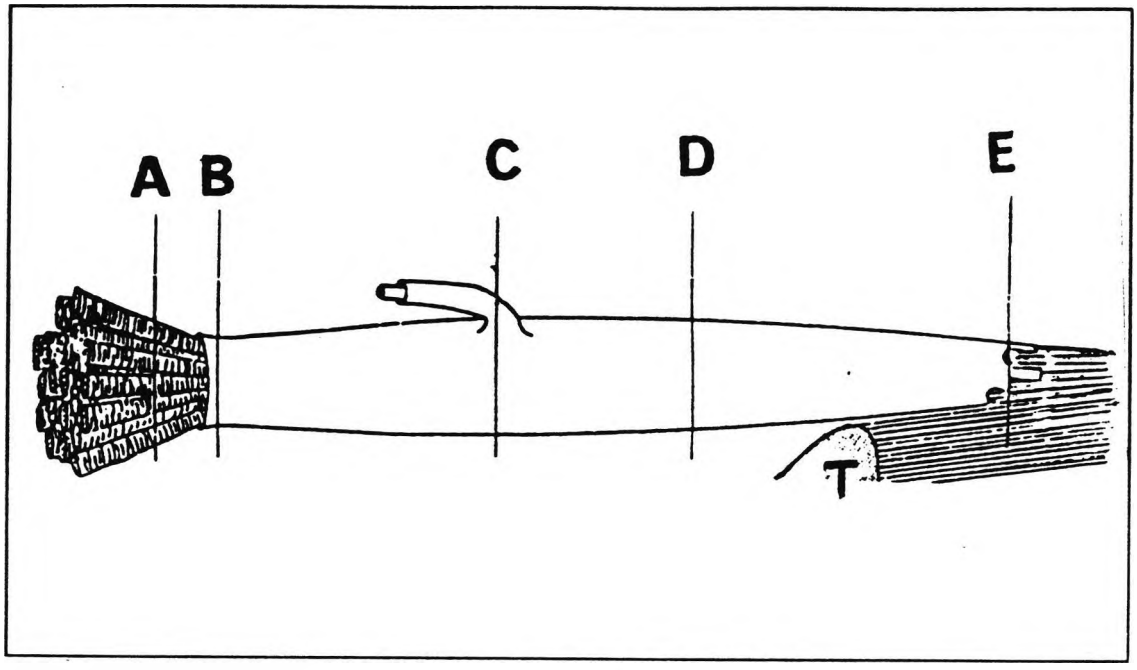


Figure 1.10 Golgi Tendon organ. Muscle fibres (A) enter the proximal capsule opening at (B). The afferent fibre (C) enters the capsule lumen, the diameter reduces distally (D) As one approaches the distal limits, the capsule diameter gradually declines. The capsular investment terminates at (E) and the emerging collagen fibrils join and blend with the fibrils of the central tendon (T). (Redrawn from Schoultz and Swett. The fine structure of the Golgi tendon organ. Journal of Neurocytol 1,1-26, 1972)

length. During stretching of a muscle the muscle spindle will create an opposing contraction . This reflex contraction increases with stretching of the muscle, but only up to a certain point, when relaxation will occur- an inverse stretch reflex. The close relationship between the axonal branches and bundles of collagen in the GTO suggests that sensory signals arise from deformation of the sensory axon during muscle activity. The mechanism responsible for converting tensile force between collagen and muscle fibres into a frequency code is uncertain. Schultz and Swett published a detailed study in 1972, where they suggested several ways in which this could be achieved. They considered and rejected the possibility that intramuscular pressures, created during muscle activity, could act upon the capsule and create hydrostatic pressure changes as it would be inconsistent with the slow adapting properties of this receptor. Schoultz and Swett concluded that the most plausible theory was that during muscle activity, the tension applied on the tendon would lead to pinching of the nerves entwined and intermingled with the collagen fibres. Such a mechanism would not be able to differentiate between active contraction of the muscle and passive stretch. This conforms with physiological studies performed on this receptor.

Although putative myotendinous receptors have been noted in the EOM they may not have the characteristics of GTO. An exception was found in non human primate where among a variety of myotendinous nerve endings a rare GTO was observed with one or two relating muscle fibres of the Felderstruktur type (Ruskell, 1990). The classical GTO form has not been reported in man.

1.3.3.2 Palisade endings

The presence of palisade endings in human EOM was first observed by Dogiel (1906) but was not confirmed until recently (Richmond et al, 1984). The interest in potential receptors at the musculotendinous junction was renewed following

reports on alteration in position sense in patients who had undergone surgery in this region of the muscle (Steinbach and Smith,1981).

ce between the nerve endings at the musculotendious junction in
et al, 1984) and the palisade endings in cat (Alvarado-Mallart
ond 1979) has been commented upon by several workers (Sodi
oth mammals, these structures derive from small myelinated
e terminal diameter seldom exceeding 5 μm. The nerve fibres
uscle insertion in small bundles along with efferent fibres. They
ndon and loop back at various distances from the site of muscle
on before they finally reach the myotendious region (Fig 1.11)
ve led to the contention that they are both palisade endings and have

? Such Structure

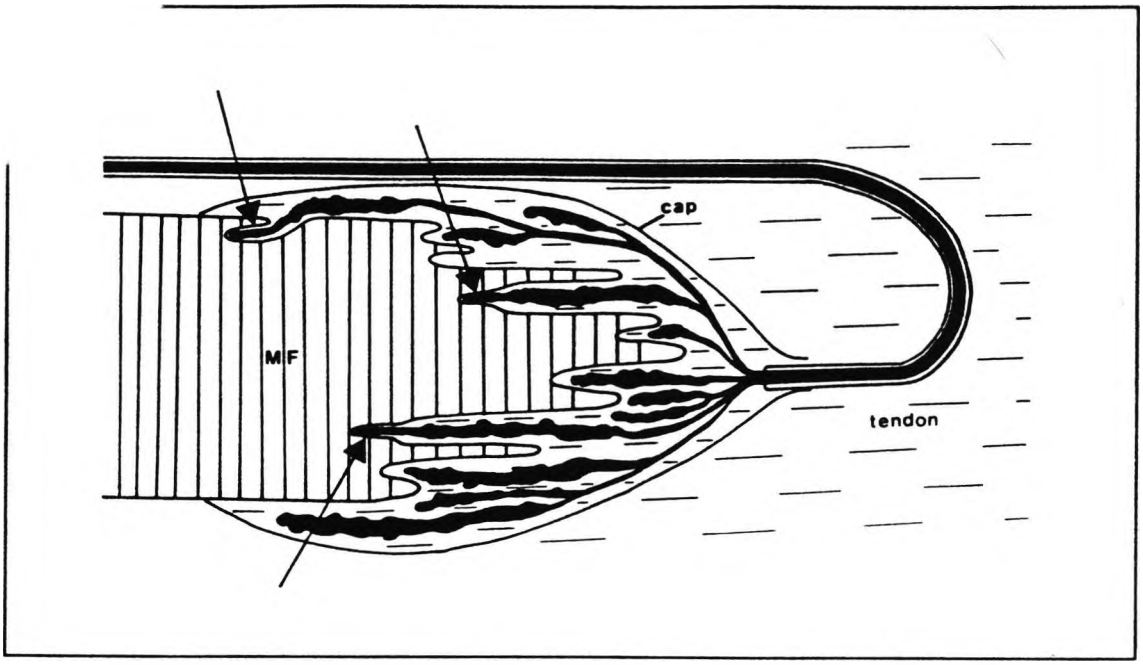


Figure 1.11 The palisade ending of cat EOM . The myelinated axon enters the capsule (cap) after looping back from the tendon and divides to form preterminal and terminal branches. The latter surround the extremity of the muscle fibre (MF) and penetrate the longitudinal infoldings formed by the muscle fibre at the myotendinous junction. Only a few of the terminal branches establish close contacts with the muscle fibre (arrows) (From Alvarado-Mallert and Pincon-Raymond. The palisade endings in cat extraocular muscles: A light and electron microscope study. Tissue & Cell. 11, 567-584,1979)

a similar function. The incidence of these receptors is not clear but they seem to be quite numerous with an irregular distribution.

In man, the physiological properties of the muscle fibre associated with myotendionous endings is still unknown. In cat , however , palisade terminals are exclusively associated with multiply innervated muscle fibres. This relationship suggest that palisade receptors are selective in their response and would only signal tension in the slow motor system. Tension produced by both the slow and fast system have been postulated to arise from collaterals of the main afferent axon, ending in tendon (Alvarado-Mallart and Pincon- Raymond, 1979). The mechanoreceptorial features of the palisade ending have led to the contention that they have a proprioceptive function. This assumption is mainly based on the ultra structural features of the nerve terminals and their failure to make contacts with the tips of the extremity of the muscle fibre. Richmond and co-workers (1984) put the location of these receptors in relation to previous clinical observation and considered them as a potential source of afferent feedback which may be removed by strabismus surgery. In contrast to the GTO, the palisade receptor has an intimate relationship with one muscle fibre only which might have functional implications for its capacity to monitor the performance of eye muscles. These receptors are found in parallel with ribbons of tedinous elements which might dampen the effect of a muscle stretch. As a result, the palisade ending might be able to distinguish between eye movements caused by contraction of its muscle in contrast to stretch of its tendon, created by contraction of the antagonistic muscle. The concept of a receptor secure from direct influence of collagen movement and hence selective in its response was first put forward by Ruskell in his study on the myotendinous cylinders (Ruskell, 1978).

1.3.3.3 Myotendinous cylinders

In mature form, these encapsulated structures are exclusive to EOM and were first found in the distal myotendinous region of the rhesus monkey (Ruskell, 1978). As with palisade endings these structures are innervated by small myelinated fibres in the region of 2.3-6 μm. However the perineurium of these nerve fibres does not contribute to the capsule formation which is made up purely of fibrocytes. The muscle fibres terminating in this complex are exclusively of the slow multiply innervated Felderstruktur type (Fig. 1.12).

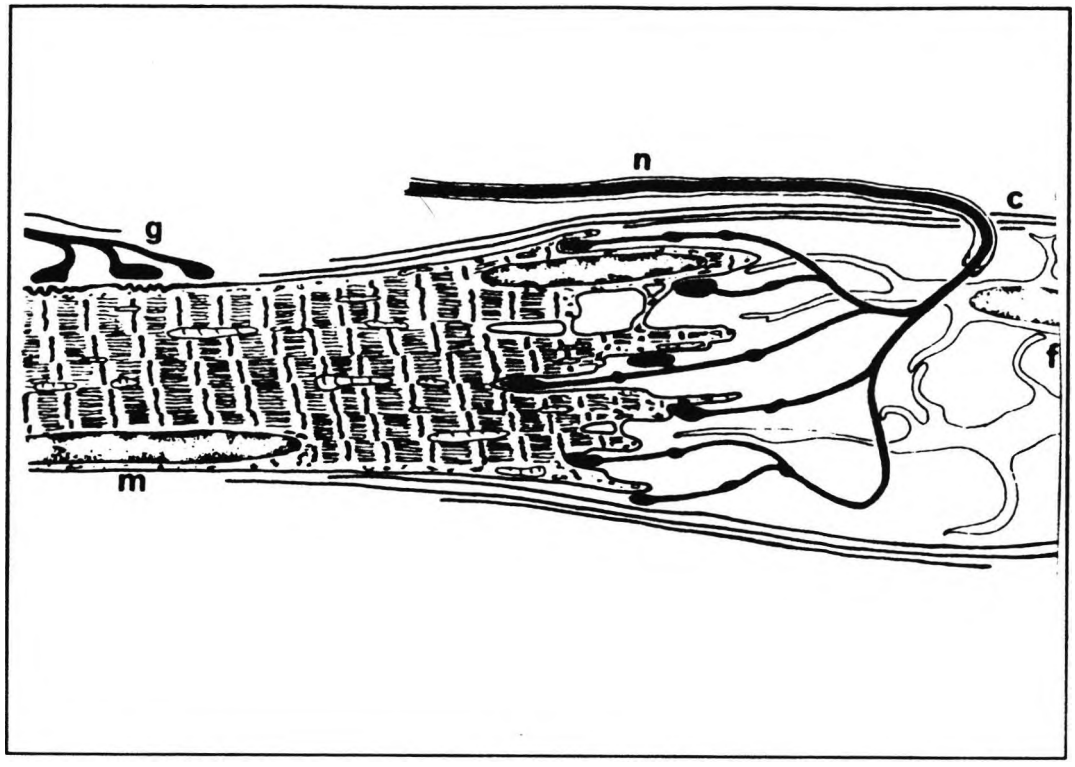


Fig 1.12 Drawing of a myotendinous cylinder. A nerve fibre (n) penetrates the cylinder capsule (c), turns, branches and makes several contacts with the muscle fibre (m). A second nerve fibre (g) forms grape endings on the muscle fibre proximal to the myotendinous cylinder. Two fibrocytes (f) of the cylinder tendon are shown in outline. (from Ruskell. The fine structure of innervated myotendinous cylinders in extraocular muscles of rhesus monkey. Journal of Neurocytology 7, 693-708,1978)

1.3.3.4 Musculo-tendinous complexes.

The ultrastructural features of the musculo-tendinous complexes described by Sodi et al (1988) do not compare with any other mechanoreceptors previously described in EOM . The main diversities lie in the size of the receptor, its capsular arrangement and mode of innervation. The total diameter of these structures lies in the region of 20μm devoid of a typical outer capsule. The discontinuous layer of flat cells surrounding the structure lies close to the sarcolemma (sometimes with a gap down to 750 nm.). They identified three subdivisions of myotendinous neural arrangements.

According to Sodi et al, it is the muscular part of this complex which is sheathed by a capsulated tendinous collar. This muscle fibre tapers off and terminates in a connective tissue component. The tendon in this region is divided into compartments by ramified cells. The nerve fibres inside this region are thought to be sensory preterminals if not terminals. This assumption is based on the ultrastructural features of the axoplasm and on comparison with previous ultra-structural data (Ruskell,1978 and 1979). A few nerve endings resembling the motor terminals of the multiply innervated tonic fibre were also found. Despite their peculiar ultrastructural features , these musculo-tendinous complexes do have some analogies to both palisade ending and tendinous cylinders. The morphological dissimilarities with the latter receptor could be species dependent. Sodi and co-workers were therefore reluctant to claim that this receptor is unique and they suggested that these complexes had a similar function as the previously described structures. The presence of efferent nerve endings is, however, more difficult to explain. They might assist the afferent system in a manner yet unknown or in a way similar to the role of the efferent supply to the intrafusal muscle fibres in the spindle.

1.4 AIM OF STUDY

There is a generous efferent nerve supply to the extraocular muscles. The diameters of these nerve fibres vary depending on the rather unique morphological features of the muscle fibres they supply. Due to the special assortment, analysis of the efferent nerve fibres in the oculomotor, abducens and trochlear nerves should include all axon diameters even down to the smallest unmyelinated nerve fibres. The relationship between small efferent nerve fibres and tonic muscle fibres was introduced by Terävainen in 1969. It is therefore reasonable to assume that earlier estimates of the motor unit, where high resolution techniques were not applied, failed to include these fibres. Furthermore, the selection of tissues used in these studies seems to be random. Potential variations in fibre content along the length of the muscle have been ignored and reference to age is often omitted. Current information on motor units in these muscles must therefore include an element of error or at best be regarded as incomplete. Despite that, the whole concept of a motor unit is fundamental to the understanding of the physiological behaviour of a muscle; detailed information regarding this unit in human EOM is not available as the foregoing has shown. One of the aims of this study is therefore to analyse the motor unit serving the different muscle fibres in the EOM. Through light and electron microscopic observations the distal branches (orbital component) of the oculorotary nerves will be analysed according to content, size and distribution of axons. From the first branch (intramuscular component) to the site of termination the axons will be traced in uninterrupted, thin serial sections.

Judging by the numerous sensory receptors in extraocular muscles, the afferent portion of the nerve must be rather substantial. The population of afferent fibres must therefore be isolated in order to exclude them from the analysis. Along with the total muscle fibre content of each muscle studied these data will form the basis for estimates of the motor unit. Tissues obtained from subjects of

various age groups will provide the means of revealing possible effects of ageing on these units.

It is tempting to assume that the existence of a precise and refined afferent system is fundamental for the oculomotor system which has to assure exact movements and co-ordination between the two eyes. However, unlike somatic musculature ,the role of both muscle spindles and tendon organs in oculomotor control is obscure. Numerous theories have been offered regarding the true function of the muscle spindle in the human EOM. However, these theories have been based exclusively on the assumption that the receptors are fully functional proprioceptors. They have been credited either as providers of conscious position sense or as participants in some subconscious nervous control of muscular contraction.

Thorough investigations of spindle morphology have revealed peculiar features, referred to above (Ruskell,1989). These observations warrant the fullest consideration because if they can be confirmed and elaborated the concept of spindle utility and extraocular muscle proprioception in general would be placed in jeopardy. Ruskell's observations were made from muscle tissue taken from human adults within a limited age span.. It therefore remains to be seen whether these peculiarities represent structural age- related changes, which would strongly suggest a developmental role for these receptors, or if they are simply caused phylogenetically. The second aim of this study is therefore to analyse the structure and potential function of muscle spindles in the EOM of human infants.

In the absence of functional muscle spindles, the oculomotor system might yet make use of other mechanoreceptors for proprioceptive information. As with somatic muscles , various structures with mechanoreceptorial features have been found at the musculotendinous junction in the EOM of various species. In man ,

the morphology and potential function of these receptors have received little attention since Dogiel discovered them in 1906. Apart from a few observations at light microscopic level (Cooper & Daniel, 1949: Richmond et al,1984) our current knowledge of these receptors comes from two conflicting ultramicroscopic observations (Mukuno,1983 and Sodi et al., 1988). The material used in these studies was obtained from adult patients. The view that differences in receptor morphology might be caused by age or phylogenetic changes is intriguing and the opportunity was taken to study infant tendon receptors. The final aim of this study is therefore to compare the structure of mechanoreceptors in the myotendinous junction of infant human EOM with those of the older age groups.

CHAPTER 2

MATERIAL AND METHODS

2.1 PREPARATION OF TISSUES FOR HISTOLOGY

Muscle samples from the superior rectus (SRM), inferior rectus (IRM), medial rectus (MRM) and inferior oblique muscle (IOM) were obtained from 19 patients of both sexes. Eight of these patients were infants, aged 1 day to 47 months (Table 2.1) while the remaining 11 were adults, aged 30 to 90 years (Table 2.2).

REF. AND AGE	MUSCLES	CAUSE OF DEATH	TIME OF FIXATION	SEX
HC 1 47 months	MRM,IRM and IOM	Congestive heart failure	3 hours PM	Female
HC 2 5 months	MRM,IRM and IOM	Died following cardiac surgery.	7 hours PM	Male
HC 3 One day	MRM	Respiratory failure.	3.5 hours PM	Female
HC 4 6 days	MRM,IRM and IOM	Died following cardiac surgery	10 hours PM	Male
HC 5 23 months	IOM	Died following MVA	7 hours PM	Male
HC 6 30 months	MRM,IRM and IOM	Accidental strangulation	1 hour PM	Male
HC 7 6 months	MRM,IRM and IOM	Anomalous coronary artery	12 hours PM	Female
HC 8 3 days	MRM,IRM and IOM	Unknown	Not recorded	Male
SUMMARY				
8 subjects 1 day to 47 months	7 MRM 6 IRM and 7 IOM	No infections, malignant processes or eye signs	1 to 12 hours PM	3 Females 5 Males

Table 2.1 Infant patient information. All muscles were removed and fixed supervised by Professor Steinbach

REF AND AGE	MUSCLES	CAUSE OF DEATH	TIME OF FIXATION	SEX
HW 4 74 Years	SRM	No infections, malignant processes or eye signs	Not recorded	Not recorded
HW 5 30 years	IOM	No infections, malignant processes or eye signs	Not recorded	Male
HW 7 70 years	IOM	No infections, malignant processes or eye signs	Not recorded	Female
HW 8 42 years	IOM	No infections, malignant processes or eye signs	Not recorded	Female
HW 9 58 years	IOM	No infections, malignant processes or eye signs	Not recorded	Not recorded
HW 11 90 years	IOM	No infections, malignant processes or eye signs	Not recorded	Female
HW 20 35 years	IOM	No infections, malignant processes or eye signs	Not recorded	Male
HW 26 76 years	IOM	No infections, malignant processes or eye signs	Not recorded	Not recorded
HW 28 73 years	SRM	Enucleated eye due to thrombotic glaucoma.	Not recorded	Male
HS1/B2 52 years	MRM	No infections, malignant processes or eye signs	Not recorded	Female
HIOM 21 years	IOM	No infections, malignant processes or eye signs	Not Recorded	Male
SUMMARY				
11 subjects 21 to 90 years	8 IOM 2 SRM 1 MRM	No infections, malignant processes or eye signs	1 to 12 hours PM	4 Females 2 Males 4 Not recorded

Table 2.2 Adult patient information. All muscles were removed and fixed by Mr Kin Wang FRCS.

Samples from both groups were obtained either following enucleation or post mortem. In the latter cases the cause of death ranged from heart failure to accidental strangulation. In those cases where the cause of death was not recorded, the absence of binocular vision abnormality or neuromuscular disease

could be confirmed in all cases with the exemption of HW5. Results obtained through samples from the latter subject were therefore excluded from the statistical analysis described in chapter 3.

After muscles were dissected from the eyes they were immersed in 5% glutaraldehyde buffered to pH 7.4 with sodium cacodylate, most of them with a post operative / post mortem delay of less than 3 hours, maximum 12. Tissue used for analysis of motor units included the thickest part of the muscle (middle third region) and in all muscles selected for this task (seven IOMs), it was possible to recognise the nerve on entry into the muscle. Muscles with complete musculotendinous junctions were selected for search of tendon receptors. Tissues were transferred from fixative to dissecting fluid (buffered sucrose) for 24 hours, washed, then immersed in a 1% solution of unbuffered osmium tetroxide for 1 hour. After washing they were dehydrated in graded mixtures of ethanol with water , initially in 50% followed by 70% and 90 % (with a duration of 20 min in each). A total of 60 min in absolute alcohol gave the final dehydration. Before embedding, the muscle samples were cleared in xylene for one hour. They were then transferred to a solution of equal amounts of xylene and Araldite for 30 minutes. At each stage the samples were left in a specimen rotator (Between 2-12 rpm.).They were finally left rotating overnight in pure Araldite resin. The specimens were embedded in Araldite- filled trays which were incubated at 60°, for 48 hours. Transverse sections of 0,75 μm and 75 nm thickness, were cut on a Reichert ultramicrotome for light and electron microscopy respectively. Semi-thin sections were stained with toluidine blue after removal of Araldite with sodium methoxide followed by a methoxide diluent, acetone and distilled water. Sections for electron microscopy were stained in a saturated solution of uranyl acetate in 70% ethanol for 20 min followed by 20 min in a solution of lead citrate in 0.1M sodium hydroxide. (0.2 g in 75 ml)

2.2 GENERAL METHODS OF OBSERVATION

2.2.1 Light Microscopy

The semi-thin sections, obtained from the various muscle samples, were analysed by the aid of a Nikkon Opthiphot-2 light microscope. This microscope had a graticule incorporated in one of the oculars so that measurements of muscle and nerve fibres could be done directly through the microscope. The instrument also had a photomicrographic attachment (microflex HFX-IIA) which was used when micrographs were required. The photomicrographic attachment was connected to a control box through a signal cord. The control box determined the exposure automatically based on the level of illumination. A 35 mm camera (Nikkon FX-35WA) was mounted on the photomicrographic attachment via an adapter (Fig 2.1).

Figure 2.1 System used for taking light micrographs. The camera is operated through the external unit which automatically selects the shutter speed based on the level of illumination.

Micrographs were not only produced for documentation of various histological observations but also formed the basis for more accurate measurements. Structures could be measured directly from the photomontage with the aid of a magnifying system with a graticule incorporated.

Such a method did, however, have limited application and proved to be very time-consuming in those cases where analysis of serial sections was required. All tasks which required analysis of serial sections were therefore attempted assisted by a computer with a purpose designed software program. The hardware consisted of a 486 processor with frame grabber and external monitor. This image analysis unit was connected to the microscope by replacing the camera

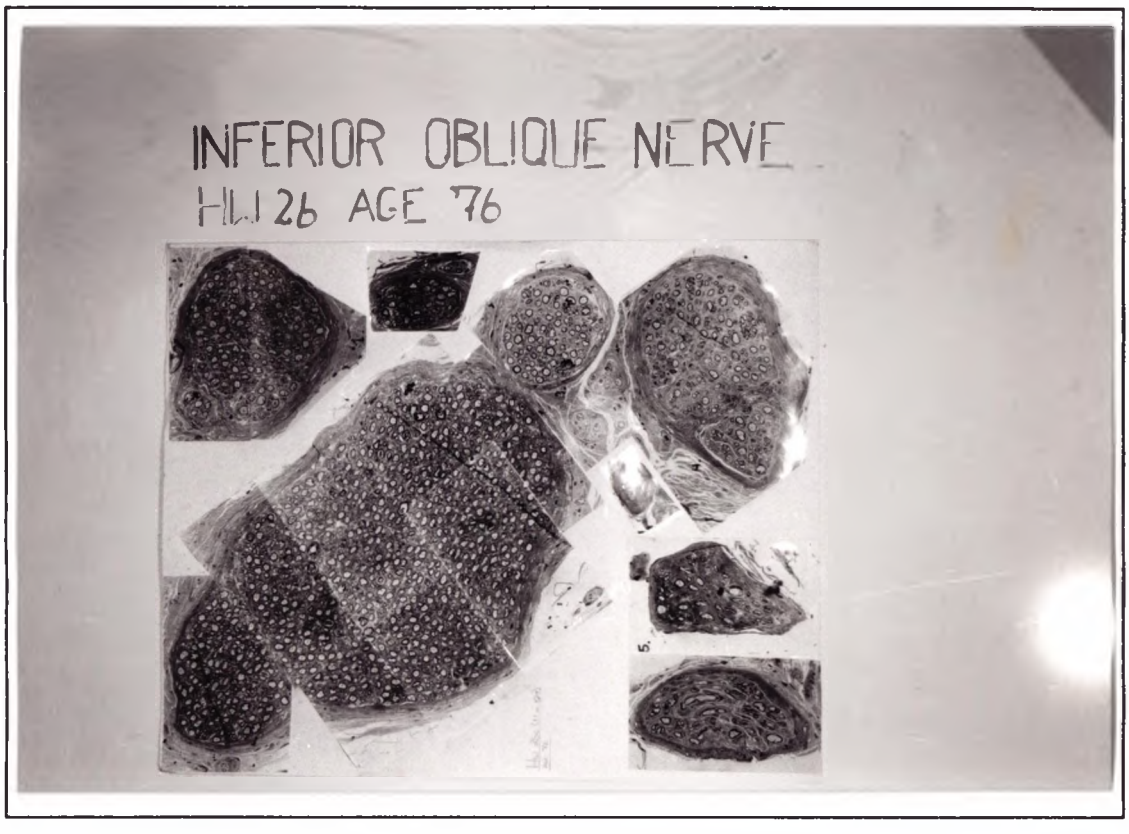


Figure 2.2 Photomontage of a nerve to the inferior oblique muscle in transverse section, stained with toluidine blue. Age of subject as indicated on the montage. Original magnification 640. (Photos of the remaining photomontages enclosed in appendix D)

unit with a CCD camera (Fig. 2.3).

The applied software was the second version of Global Lab Image which is developed by Data translation and is based on Microsoft windows. The program automatically detected, counted and measured all objects within a set region of interest and hence made it easier to trace the individual structures. One histological section could be compared with that following, either by superimposing the two images on the external monitor or simply by comparing the two prints . The program could also differentiate between different types of objects based on variation in size, shape, intensity, orientation or other parameters. This option was used to exclude muscle fibres from the region of

Figure 2.3 System used for image analysis. Signals from the microscope are sent to the computer for histological analysis while the image of the section is displayed on the external monitor.

interest during analysis of axons and vice visa (Fig. 2.4). The program could also detect deformed fibres/cells by measuring along more than one axis over the cross sectional surface of the object of interest.

The program could be calibrated by referencing an object of known dimension, in this case the microscope graticule. By using this precise calibration all measurements were specified in units of μm. Statistical representations of the measurements of selected objects were produced by transferring the numerical data to a spread sheet, in this case Microsoft Exell.

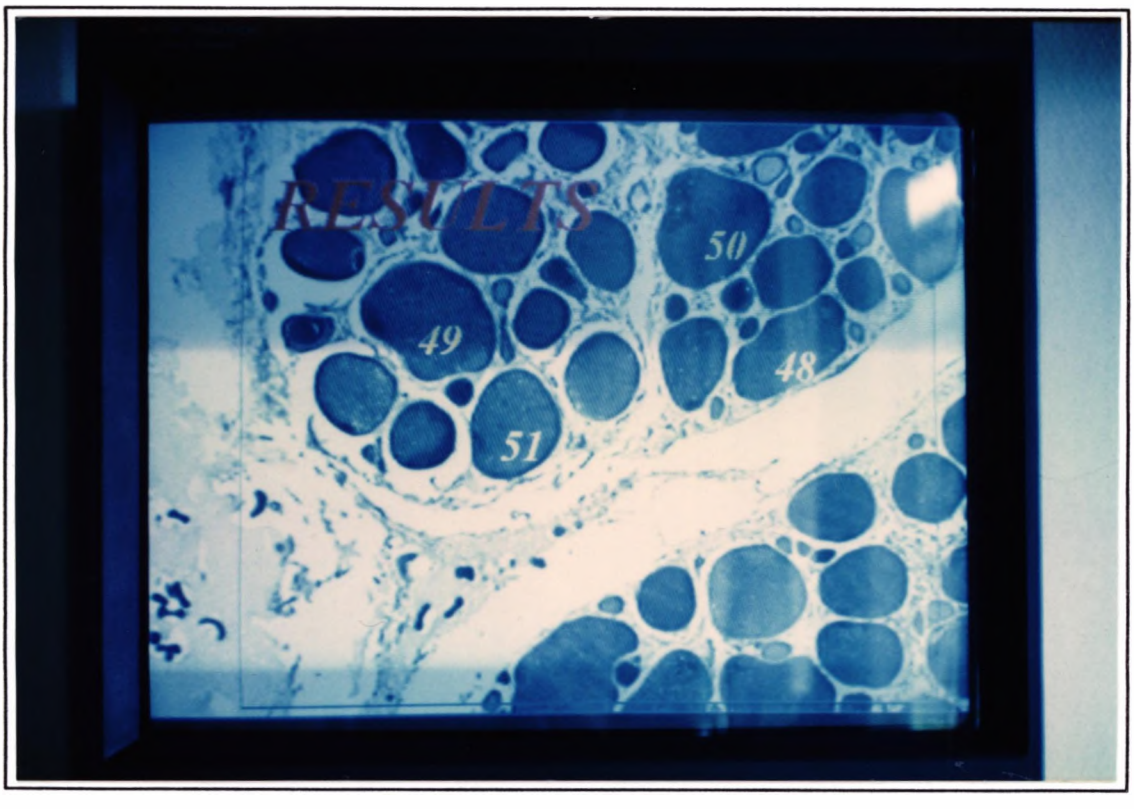


Figure 2.4 Photo of the screen monitor to illustrate how the data program measures and counts all fibres within a set region of interest. The program is also capable of selecting fibres after specified parameters. In this example fibres 48 to 51 have been selected because they exceeded a set diameter.

2.2.2 Electron Microscopy

Ultrathin sections were cut using a diamond knife and mounted on unfilmed 200 mesh copper grids . These grids had a diameter of 3.05 mm while each square in the grid measured 97 by 97μm, separated by 28 μm wide bars .

Low magnification was used during analysis of unmyelinated nerve fibres. The number of unmyelinated fibres found in each square was recorded directly from the microscope screen, and the number of fibres covered by the gridbars was estimated. Variations in number and distribution of fibres within each square were taken into consideration when the number of fibres covered by gridbars was estimated.

Analysis and microphotography was done by the aid of a Jeol 100 CX electron microscope (Fig 2.5). The Jeol 100 CX is an analytic instrument in the sense that a variety of analysis techniques can be used on the same instrument. The technique used in this study was exclusively transmission electron microscopy.

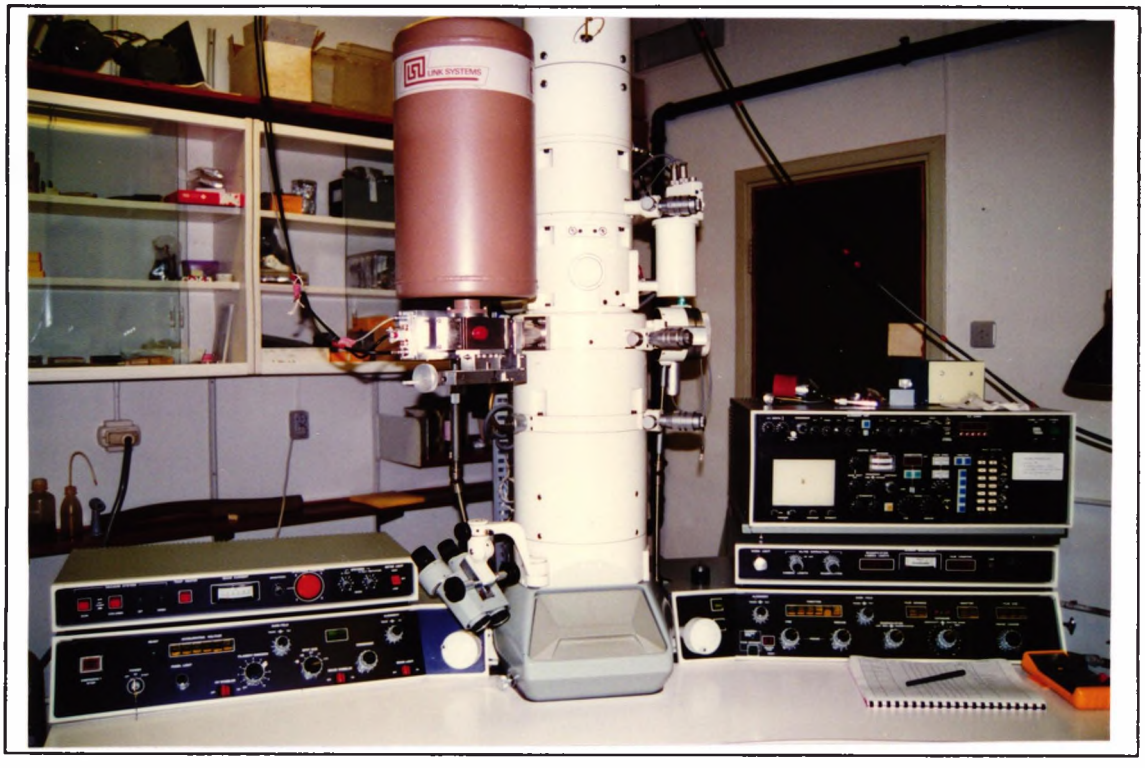


Figure 2.5 Electron microscope, type Jeol 100 CX .

2.3 ANALYSIS OF NERVE AND MUSCLE TISSUE

2.3.1 Analysis of Nerve/Muscle Fibre Ratios.

Seven inferior oblique muscles, with attached nerves, were selected from the group of adult subjects. Samples were obtained from both sexes with an age range from 30 to 90 years (Table 2.3).

After dissection and preparation, complete semi-thin transverse sections of the 7 nerves were cut. Sections containing the largest cross sectional diameters were

PATIENT	AGE	TYPE OF SAMPLE	SEX
HW 5	30 years	Nerve	Male
HW 7	70 years	Nerve	Female
HW 8	42 years	Muscle and nerve	Female
HW 9	58 years	Muscle and nerve	Not recorded
HW 11	90 years	Muscle and nerve	Female
HW 20	35 years	Nerve	Male
HW 26	76 years	Muscle and nerve	Not recorded
SUMMARY			
7 Patients	30 to 90 years	7 nerves 4 muscles	2 Males 2 Unknown 3 Females

Table 2.3 Details of patient material used in the analysis of motor units of the inferior oblique muscle.

obtained from 4 of the corresponding muscles. The three remaining muscles were incomplete and therefore were excluded from the analysis.

A photomontage was produced for each of the muscles used to ensure a correct topographical registration of the various regions during counting (See appendix C). A similar montage was made for each of the nerves but this time with a higher resolution, total magnification of 640 (Fig. 2.2). The latter were used for analysis of the large myelinated nerve fibres. All measurements were made along the shortest axis of fibres. The diameter of the nerve fibres (measured from the montage with the aid of a magnifying system with an eyepiece graticule) included the axon and its surrounding myelin, regardless of the presence of Schmidt- Lanterman clefts. Random samples of the obtained data were later verified by using data based image analysis (described on page 62). Following semi-thin sectioning, the tissue block was trimmed and ultrathin sections (70-100 nm) were cut using a diamond knife. These sections were mounted on copper grids. All analysis of the small myelinated and unmyelinated axons was done from the screen of the electron microscope.

2.3.2 Estimates of the Number of Branches of Single Intramuscular Nerve Fibres

From the same material used for motor unit estimates, axons were traced in successive serial sections over a distance varying from a few hundred microns to several millimetres . From their termination on the muscle fibre , the axons were traced backwards to the point of fusion with another branch of the motor unit. The topographical distribution of muscle fibres belonging to the same unit within the muscle was recorded. Initially this was done manually by tracing as many axons as possible, assisted only by simple drawings of the successive sections. This method proved to be time consuming and inaccurate. Tracing of nerve

fibres was therefore attempted using the computerised image analysis system described on page 62.

2.3.3 Muscle Spindles

Twenty muscle samples were obtained from the 8 infant subjects, none of which had a record of orbital disease. Seven out of the twenty samples were from the medial rectus muscles, six from the inferior rectus and seven from inferior oblique muscles (as indicated in table 2.1). Both sexes were represented, ranging from 1 day to 47 months of age. None of the patients had a history of any ocular involvement.

Most specimens were taken from the distal part of the muscle, 3 to 10 mm in length including part of the tendon in a majority. Those without tendon had most of the motor end plate region included. The full width of the muscle was present in each case.

All spindles previously found in human EOM have exceeded 50 μm. in length (Cooper and Daniel, 1945: Merrillees et al, 1950 and Ruskell, 1989). Semithin sections were therefore initially collected at intervals of 50μm. When a spindle was found, uninterrupted serial sections were then taken. In some samples frequency was sufficient to require serial sections throughout the whole muscle. Measurements of the intrafusal fibres, the capsule and surrounding extrafusal fibres were taken through the light microscope by aid of a calibrated microscope eyepiece graticule. The data obtained was later confirmed by using data analysis.

2.3.4 Tendon Receptors

Muscle tissue which had the distal insertion intact was found in both age groups. Samples from 12 muscles were obtained from 4 adult and 6 infant patients of both sexes, aged 3 days to 74 years (Table. 2.4).

Sections were cut through tendon with 50 μm intervals until nerves were identified. Serial sections were obtained after identification of neural elements. In some cases serial sections were required through the whole specimen. Ultrathin sections for electron microscopy were obtained with regular intervals. These sections were used to search for potential neural elements beyond light microscopic resolution.

PATIENT	AGE	MUSCLE	SEX
HC8	3 DAYS	MRM, IOM	MALE
HC 4	6 DAYS	IRM	MALE
HC 2	5 MONTHS	MRM IRM	MALE
HC7	6 MONTHS	IRM	FEMALE
HC6	30 MONTHS	MRM	MALE
HC 1	47 MONTHS	IRM	FEMALE
HIOM T1/T2	21 YEARS	IOM	MALE
HS1/B2	52 YEARS	MRM	FEMALE
HW 28	73 YEARS	SRM	MALE
HW 4	74 YEARS	SRM	NOT RECORDED
SUMMARY			
10 Patients	1 Day-73 Years	4 MRM, 4 IRM, 2 SRM, 2 IOM	3 Females 6 Males

Table 2.4 Source of the tissues used in the tendon receptor study.

CHAPTER 3

RESULTS

3.1 GENERAL MUSCLE MORPHOLOGY

Light microscopic observations revealed many of the distinct structural diversities of human EOM, previously described by others (section 1.1.4) . The rich amount of connective tissue was most apparent in transverse sections where the perimysium divided fibres into bundles by forming intramuscular septa. The pronounced endomysium separated the individual, roughly circular- shaped, muscles fibres. Not even in the most populated central regions of the muscle did these fibres take up the polygonal appearance which is so often seen in transverse sections of skeletal muscle fibres. Numerous blood vessels and nerves were embedded in the perimysium and endomysium. The capillary network was most pronounced towards the periphery of the muscle, in the zone facing the orbit. Complete transverse sections through the belly of these muscles revealed large individual variations in both size and fibre distribution. Variation in muscle cross sectional area was most pronounced in the oblique muscles (Fig 3.1). The cross sectional area of 5 inferior oblique muscles varied from being elongated and strap like (HW 7,8 and 26) to close to circular (HW11). Topographical variations did not seem to influence the layered organisation of muscle fibres. All muscles displayed a distinct outer orbital layer adjacent to the periorbita (Fig 3.3) and an inner global layer opposite the eye. The regional distribution of muscle fibres was, however, not found to be as regular as often indicated in the literature (Locket, 1968 ; Nunomura et al,1984) where the orbital layer is claimed to consist of relatively small fibres surrounding a global region of larger but more variable fibre sizes (Peachey,1971).

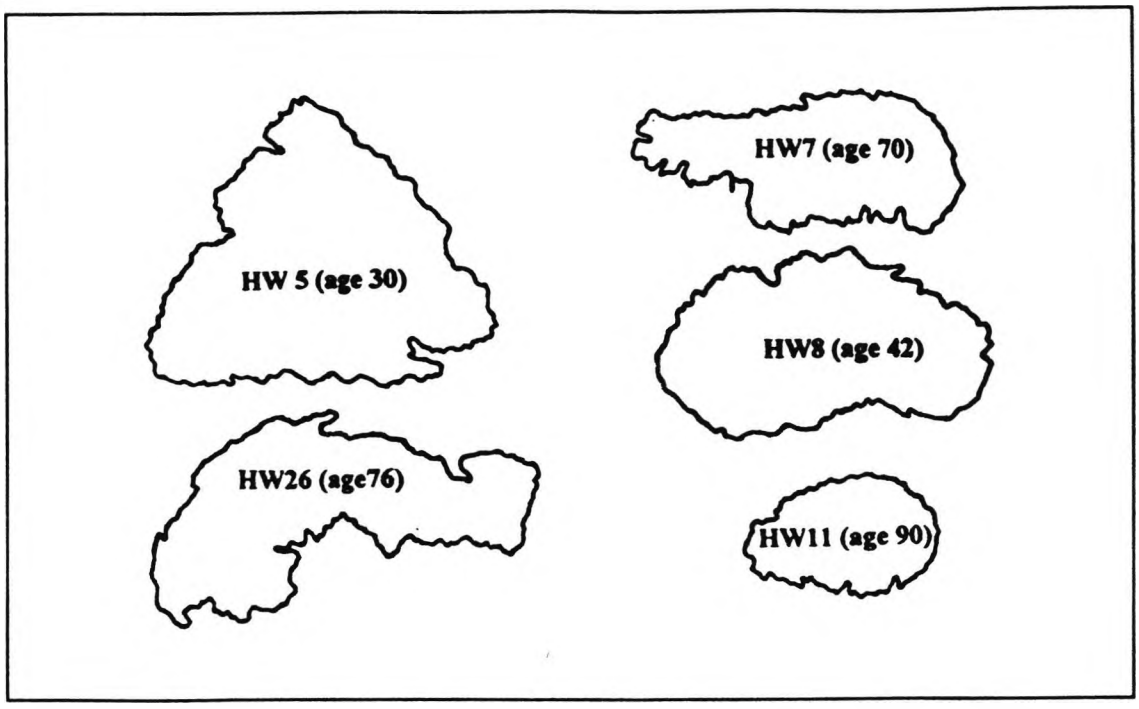


Figure 3.1 Profiles of transverse sections of 5 Inferior Oblique muscles taken from their thickest part (the region indicated in figure 3.2).

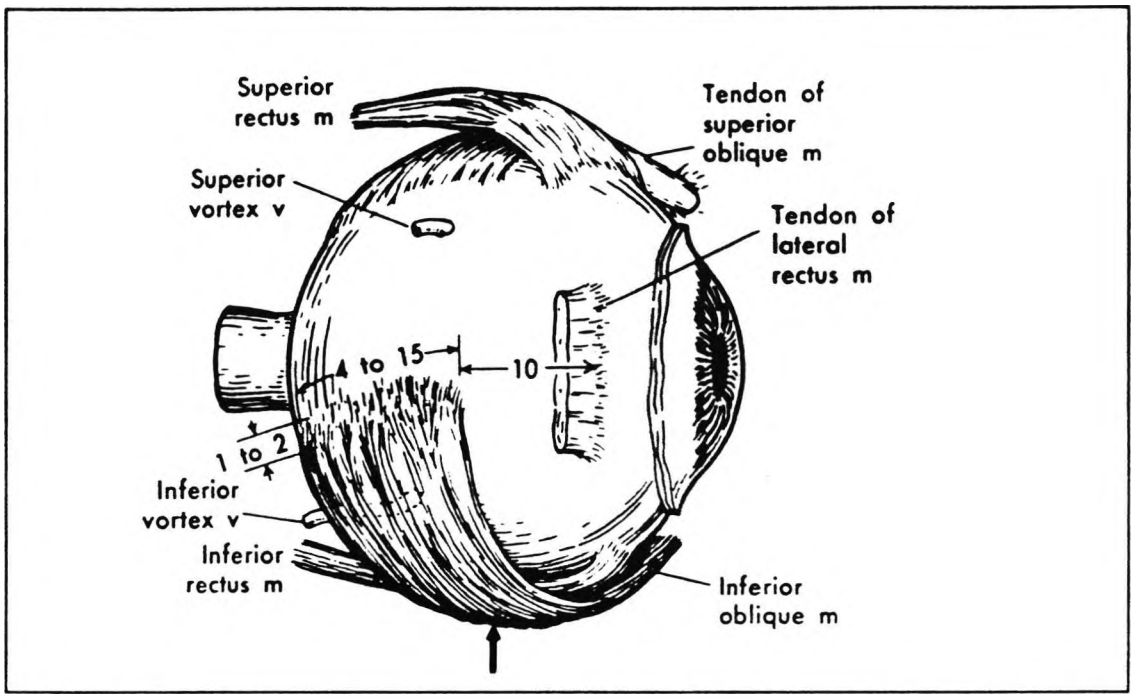


Figure 3.2 Schematic representation of the course of the inferior oblique muscle. The nerve branch to the inferior oblique muscle enters the muscle from the bulbar side just after the muscle passes lateral to the inferior rectus muscle. This region usually coincides with the thickest part of the muscle (arrow). Measurements in millimetres. (From G.K. von Noorden.) In Binocular vision and ocular motility. Ed. G.K. Von Noorden. Mosby Company, 1990. St. Louis.

Many of the fibres in the orbital layer stained densely and a number of them possessed central nuclei. In the majority the fibre diameter ranged from 7μm to 25μm but numerous larger fibres with diameters exceeding 50 μm were also present. Furthermore, densely staining fibres were not a feature exclusive to the global layer. Similar fibres were seemingly randomly distributed in the central region of the muscle (Fig. 3.3).

Under high magnification the mitochondrion content was found to be either evenly distributed in-between the myofibrils, concentrated in clusters or displaced towards the periphery forming a ring around the central mass of contractile material. Sparse amounts of sarcoplasmic reticulum gave the myofilaments little individual separation and ill-defined boundaries which was

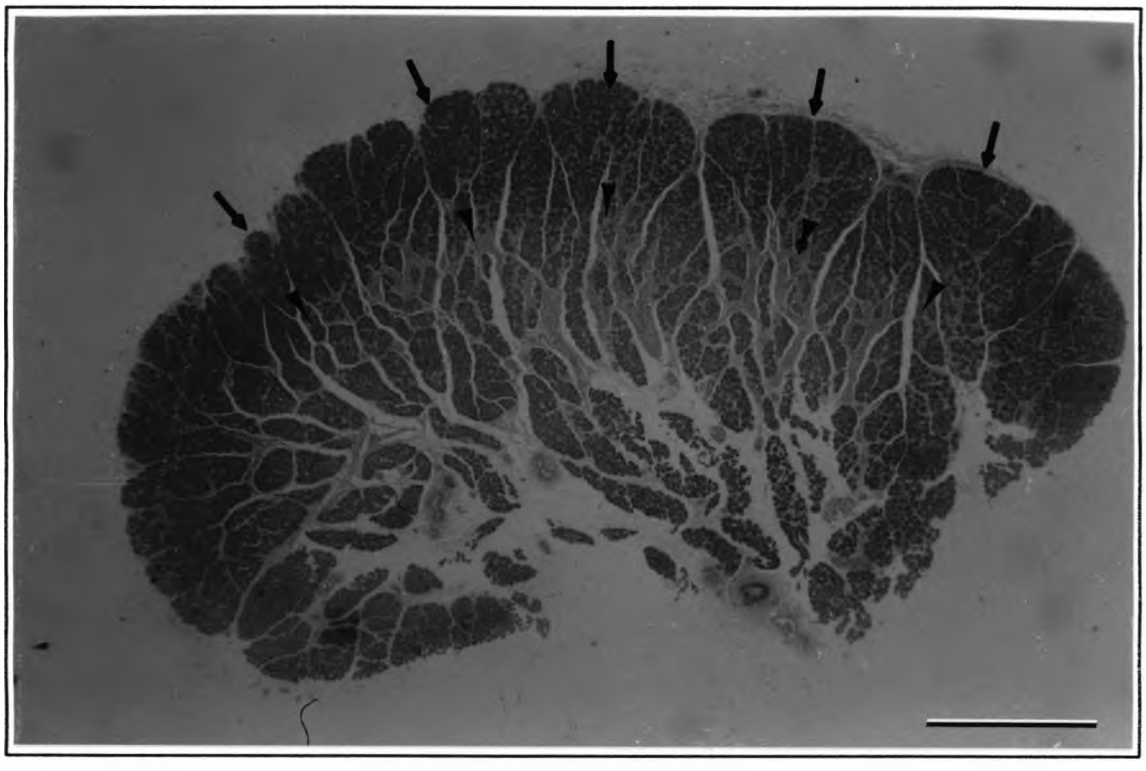


Figure 3.3 Transverse section through the belly of a inferior oblique muscle (MEP region) showing variation in fibre diameter and absorption of stain in both the orbital (arrows) and global (arrowheads) layers. Stained with toluidine blue. Age of subject is 5 months.
Marker indicates 500μm.

evident even at light microscopic level (Fig. 3.4). This limited content of both the transverse tubular system and sarcoplasmic reticulum stood in contrast to the well-developed membranous systems of the pale staining fibres which were aggregated in the axial zone of the muscle. These fibres had a fine stippled appearance, well delineated myofibrils and peripherally placed nuclei. In the middle third region of the muscle, axons were frequently found to terminate on such muscle fibres, displaying motor end plates with terminal boutons clearly indenting the sarcolemma (Fig 3.5). The majority of these fibres had a diameter which fell within the region of 30μm to 60μm while some fibres exceeded 70 μm in diameter. The two distinct fibre types were found to have features consistent with the fibres previously described as Felderstruktur and Fibrillenstruktur.

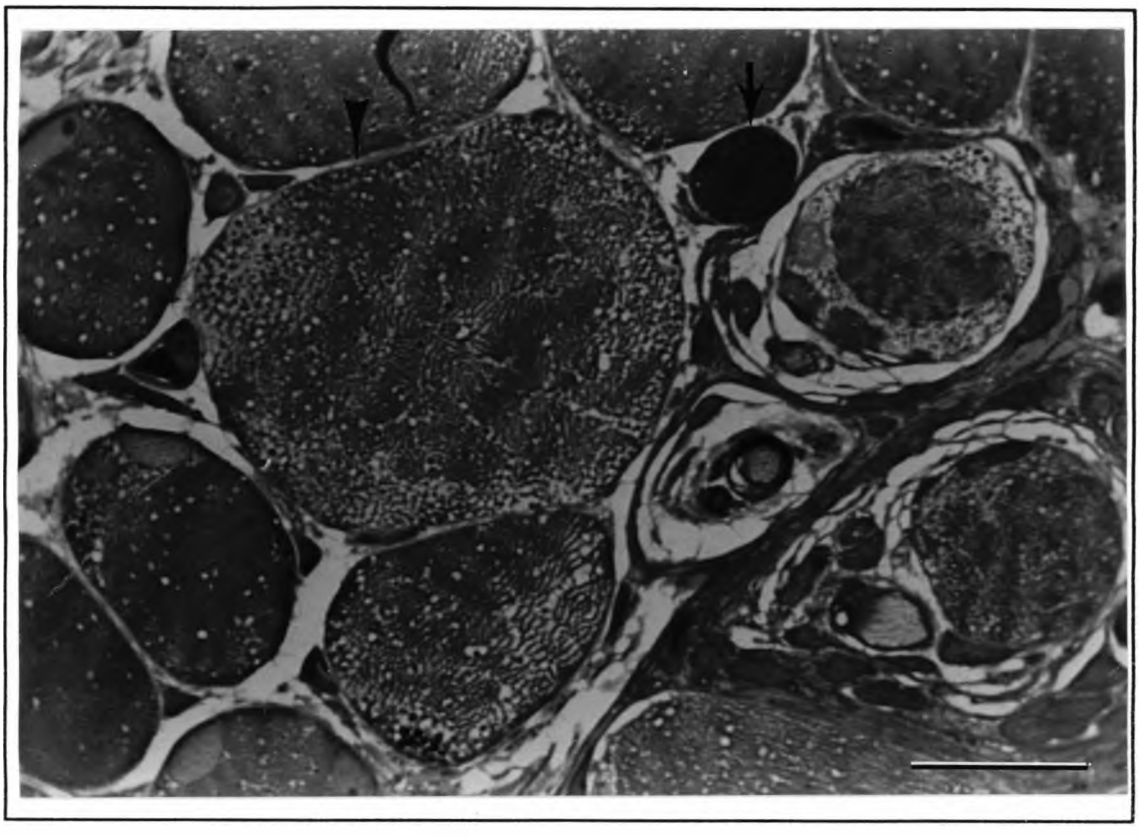


Figure 3.4 Light micrograph of Felderstruktur fibre (arrow) and Fibrillenstruktur fibre (arrowhead) in transverse sections. Diversities in fibre diameter and structural arrangement of the sarcoplasmic reticulum is evident. Sample obtained from a 30 year old male and stained with toluidine blues. Marker indicates 20 μm.

It was clear that further subdivisions of the fibre population on morphological grounds was possible as described in section 1.1.4. However, classification of fibres was not among the primary aims of this study and further subdivision of these fibres was therefore not attempted.

Morphology was found to vary between samples from young and old patients. In specimens obtained from infant patients, numerous muscle fibres with central areas free of contractile material were found aggregated in various regions of the muscle. Unlike the centrally located nuclei, occasionally observed in transverse sections of mature Felderstruktur fibres, the diameters of these areas, assumed to be nuclei, were large with ill-defined boundaries and left only a restricted ring

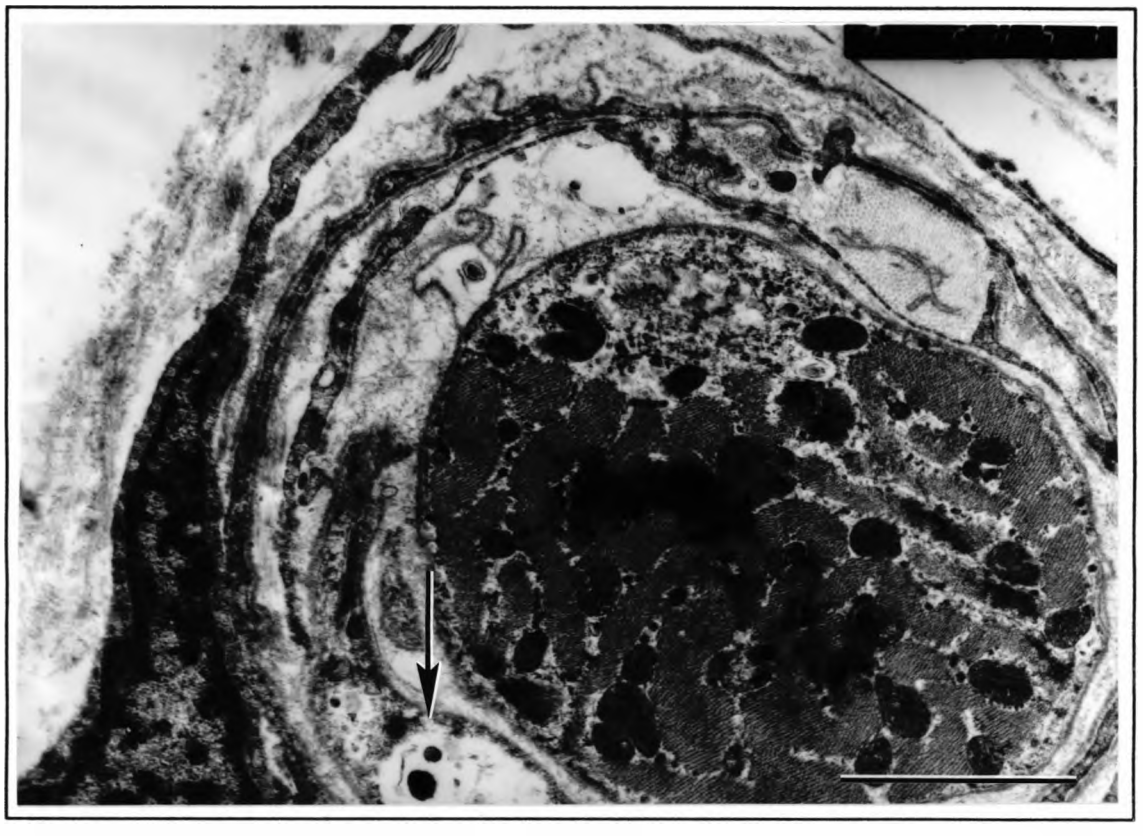


Figure 3.5 Electron micrograph showing transverse section of a Fibrillenstruktur fibre at the site of the neuromuscular junction. The presynaptic axon (arrow) contains numerous vesicles and mitochondria. Age of subject 30 years. Marker indicates 5 μm.

of contractile tissue at the periphery of the fibre (Fig. 3.6).
Serial sections presented a repetitive pattern not dissimilar to the nuclear arrangement of the intrafusal nuclear chain fibres with short inter-nuclear distances. The sizes of the fibres containing such nuclei spanned over a large range of diameters and could be found in all infant material used in this study.

In the aged material, abnormal orientation of myofilaments could be found in the periphery of some muscle fibres. In transverse sections, accumulation of myofilaments in the central region of the muscle fibre gave the expected fine stippled appearance but the peripheral part, however, displayed cross striations as

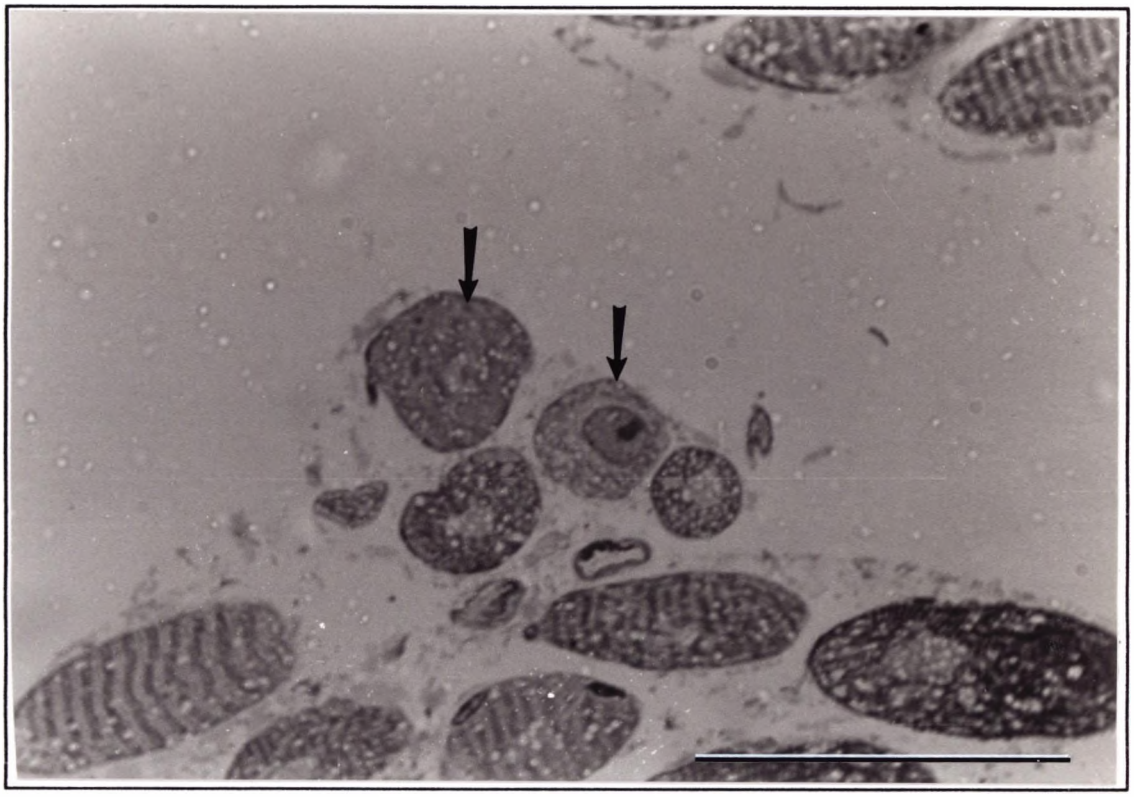


Figure 3.6 Light micrograph of transverse section through a muscle obtained from a human infant (five months old). Several muscle fibres have large centrally placed areas free of contractile material (arrows). Stained with toluidine blue. Marker indicates 50 μm.

one would see in a longitudinal section (Fig. 3.7). In some of these abnormal fibres there was a narrow cleft separating the two types of myofilaments. The location of the fibres seemed random but their occurrence increased with the age of the specimen. These fibres, identified as Ringbinden fibres (Muhlendyck & Ali, 1978), were found in all muscles from the adult group and ranged from only a few up to more than hundred in a single section. They were not observed in muscle samples from subjects younger than 45 years.

Electron microscopic observations of the adult material revealed occasional fragmentation and loss of myofibrils. In some areas the contractile elements were replaced with dense material assumed to be lipofuscin.

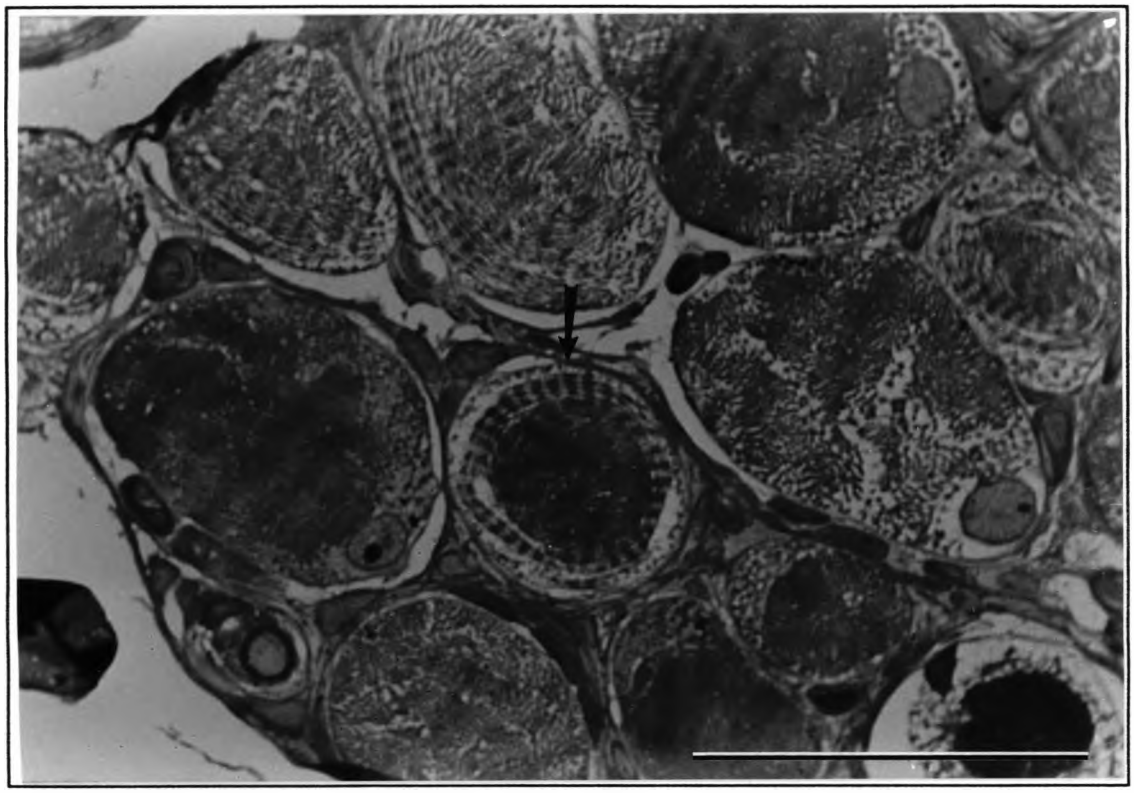


Figure 3.7 Light micrograph showing transverse section of a Ringbinden fibre (arrow). The central area is transversely orientated with a ring of cross striated muscle tissue towards the periphery. The sarcomere pattern of the transverse area appears conventional with prominent A bands and Z lines. Age of subject is 90 years. Stained with toluidine blue. Marker indicates 50 μm.

3.2 MOTOR UNITS OF THE INFERIOR OBLIQUE MUSCLE.

With one exception, fibre counts of the nerves innervating the IOMs showed moderate individual variation. In six nerves the number of myelinated axons ranged from 2424 to 3488 with a mean value of 2886. A seventh nerve (HW5) was substantially different with a remarkably high number of more than 8000 fibres. The data obtained from this nerve and the corresponding muscle were not used in the statistical analysis due to this apparently aberrant result and to the patient's unknown case history.

Using the electron microscope, in order to identify the unmyelinated fibres, axons were found in small bundles of 1-5 axons, surrounded by a single Schwann cell (Fig 3.9). Nerves contained between 117 to 270 of these bundles which could be found scattered throughout the nerve . With the exception of the nerve labelled HW5, the number of unmyelinated axons ranged from 368 to 658, with a mean of 465. This constitutes 16.1% of the fibres with a range of 10.2% to 19.3 %. The ratio between myelinated and unmyelinated fibres is hence fairly uniform and does not seem to be affected by fibre quantity. This is exemplified by the nerve HW5 (Fig. 3.8) where the ratio falls within the normal range span (17.8% unmyelinated fibres) despite a clearly abnormally high fibre content (a total of 9769 nerve fibres).

Four of the 7 nerves were selected for analysis of the spectrum of nerve fibre diameters. Using light microscopy the spectrum of myelinated nerve fibre diameters was found to have a bimodal distribution. The first peak occurred between 2 and 3μm while the second peak showed a larger span, from 6 up to 10 μm. The myelinated fibres had a diameter from 1 to 16μm. Further analysis using the electron microscope, provided a third peak which ranged over

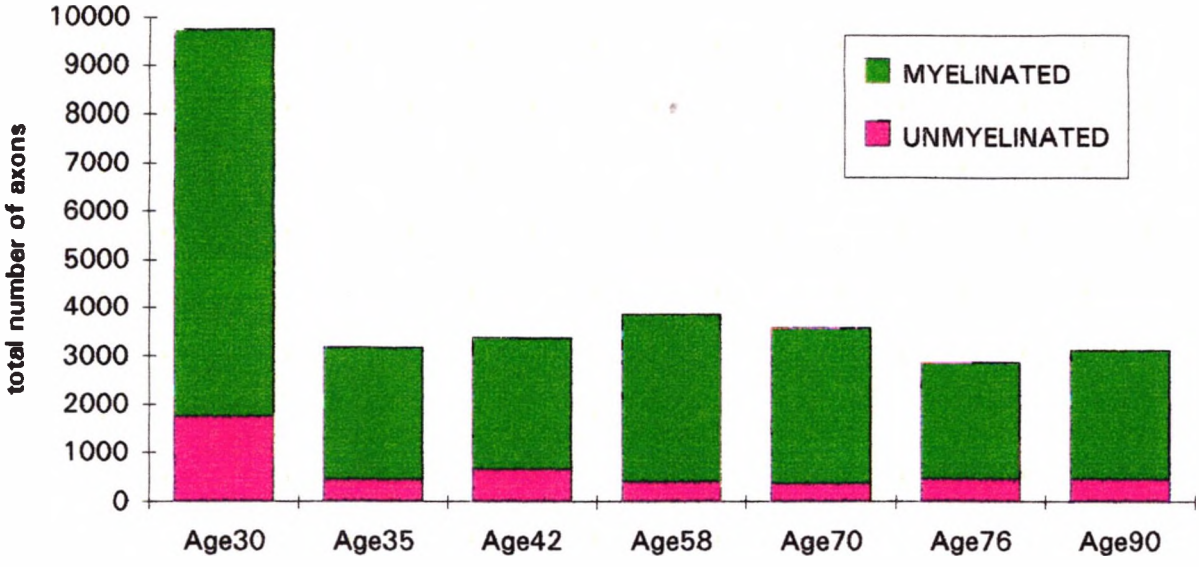


Figure 3.8 Histogram showing the total number of nerve fibres in 7 nerves to the inferior oblique muscle. Age of subject as indicated.

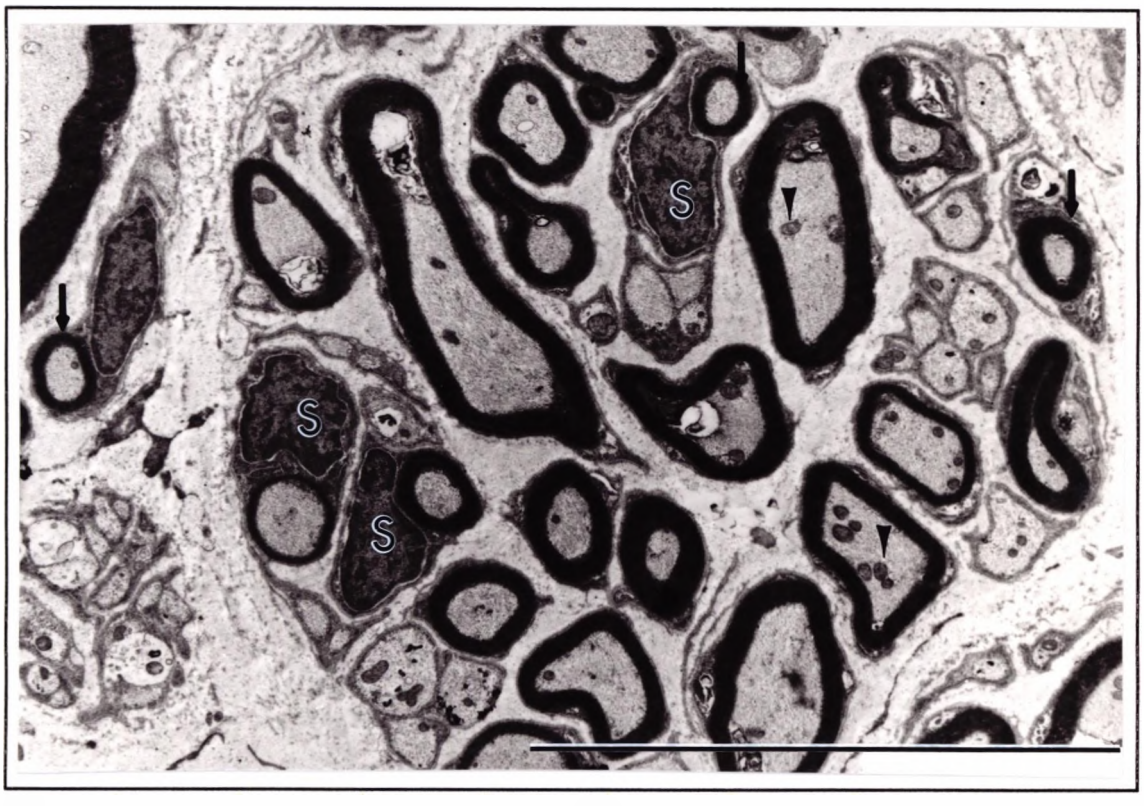


Figure 3.9 Electron micrograph of a transverse section of part of the nerve to IOM showing small myelinated (arrow) and unmyelinated nerve fibres. Schwann cell nuclei (S). Mitochondria (arrowheads) . Age of subject is 30 years. Marker indicates 10 μm.

3.13). The uniformity in fibre distribution between the various nerves (with exception of nerve HW5) is illustrated in figure 3.14.

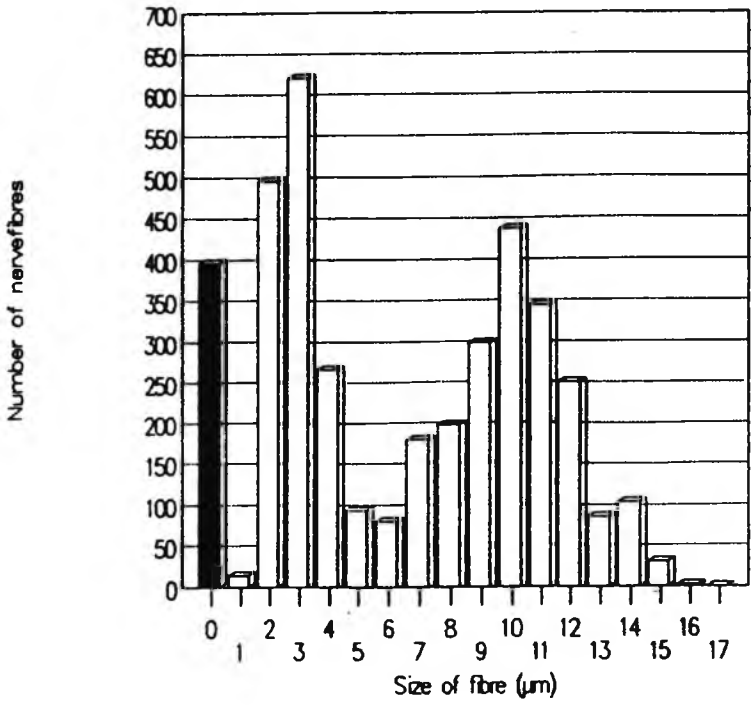


Figure 3.10 Histogram showing the diameter spectrum (µm) in the nerve (HW9) to the inferior oblique muscle (age of subject 58). Black bar represents unmyelinated nerve fibres

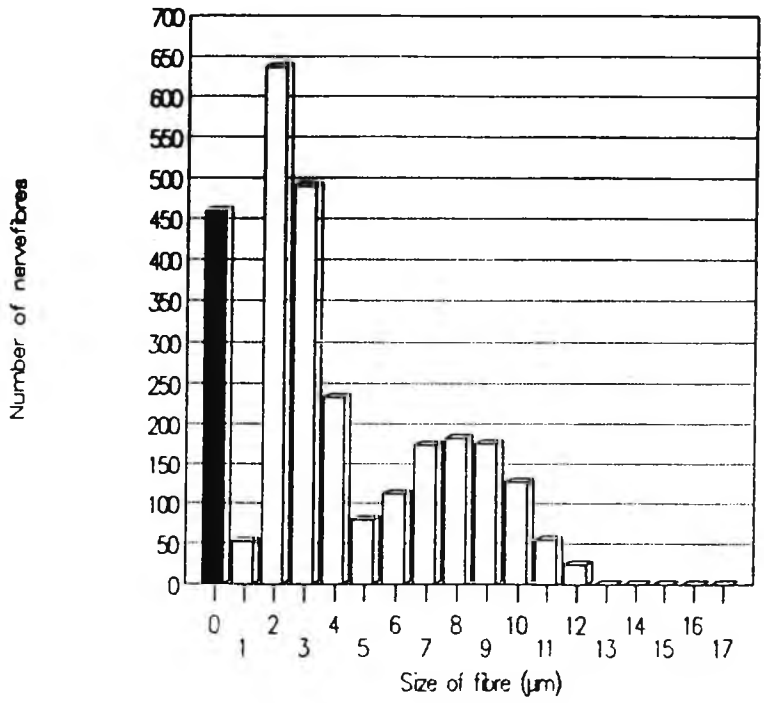


Figure 3.11 Histogram showing diameter spectrum (µm) in the nerve (HW26) to the inferior oblique muscle (age of subject 75). Black bar represents unmyelinated nerve fibres

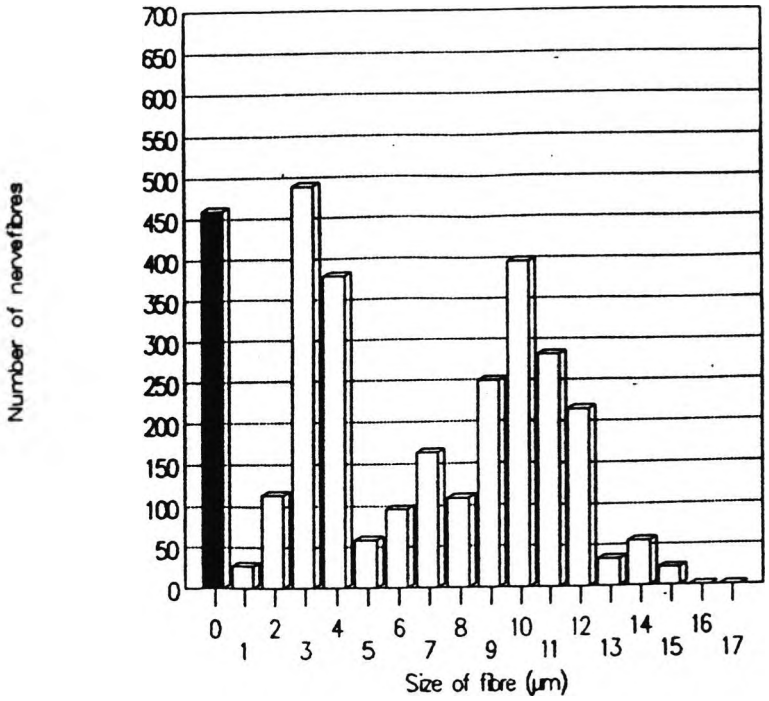


Figure 3.12 Histogram showing the diameter spectrum (μm) in the nerve (HW11) to the inferior oblique muscle (age of subject 90). Black bar represents unmyelinated nerve fibres

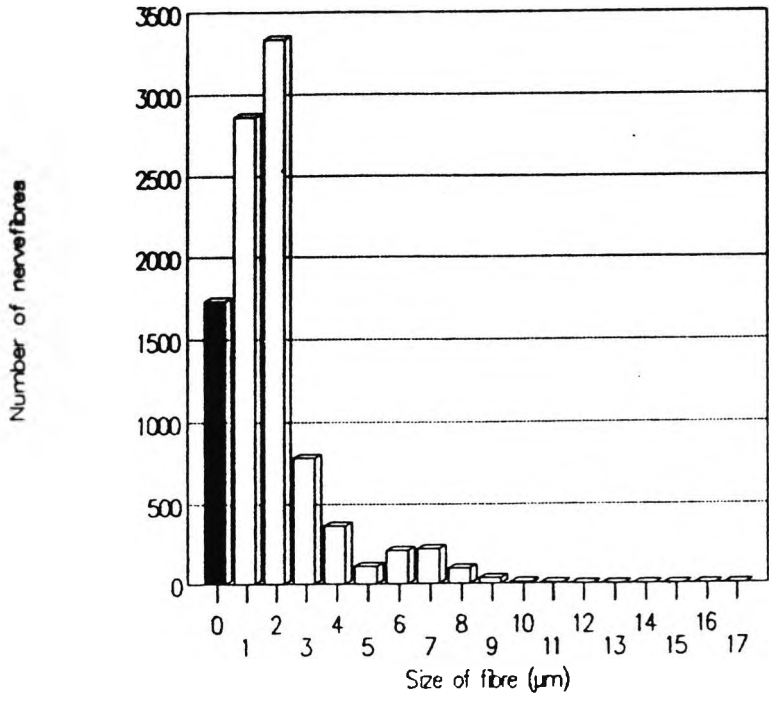


Figure 3.13 Histogram showing the diameter spectrum(μm) in the nerve (HW5) to the inferior oblique muscle (age of subject 30). Black bar represents unmyelinated nerve fibres The scale in this histogram is inconsistent with the previous 3 due to the aberrant fibre content.

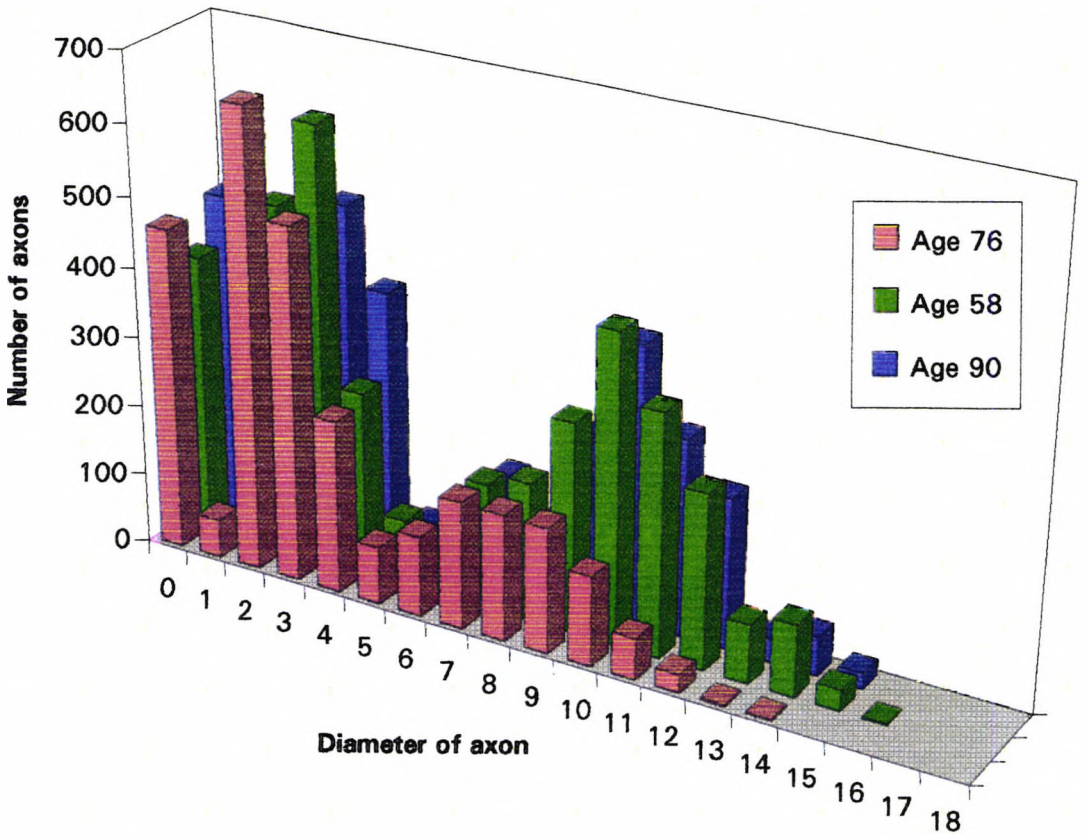


Figure 3.14 Histogram showing consistency in the nerve fibre distribution in the nerves selected for fibre diameter(μm) analysis. The nerve labelled HW5 is not included. Age of subject as indicated.

As the corresponding muscle of two of the specimens was incomplete and a third specimen was unsuitable (see above) only four preparations were used to determine nerve/muscle fibre ratios. The total number of muscle fibres in the 4 remaining muscles varied from 5875 up to 16152 with a mean value of 10966. The highest number of fibres was found in the IOM of a 42 year old patient (HW8) while the muscle with the lowest number was obtained from a 90 year old (HW11), both females (Fig. 3.15). The ratio between the number of muscle fibres and corresponding nerve fibres in the 4 inferior oblique muscles (HW7,8,11 and 26) was found to range from 1.87 up to 4.75 with a mean of 3.33.

Estimates of single motor units was attempted by tracing efferent fibres in serial sections along their length from their entry into the muscle to the respective motor endplates. Once inside the muscle the main nerve trunk gave off numerous branches over a rather short distance. Sections obtained only 1000 to 1500 μm in either distal or proximal direction from the neuromuscular hilum displayed mostly small nerve fascicles of less than 100 fibres. These gave off fine branches, minimally containing a single fibre which often passed forward oblique to their terminal sites. Nerve fibres serving the same muscle fascicle could

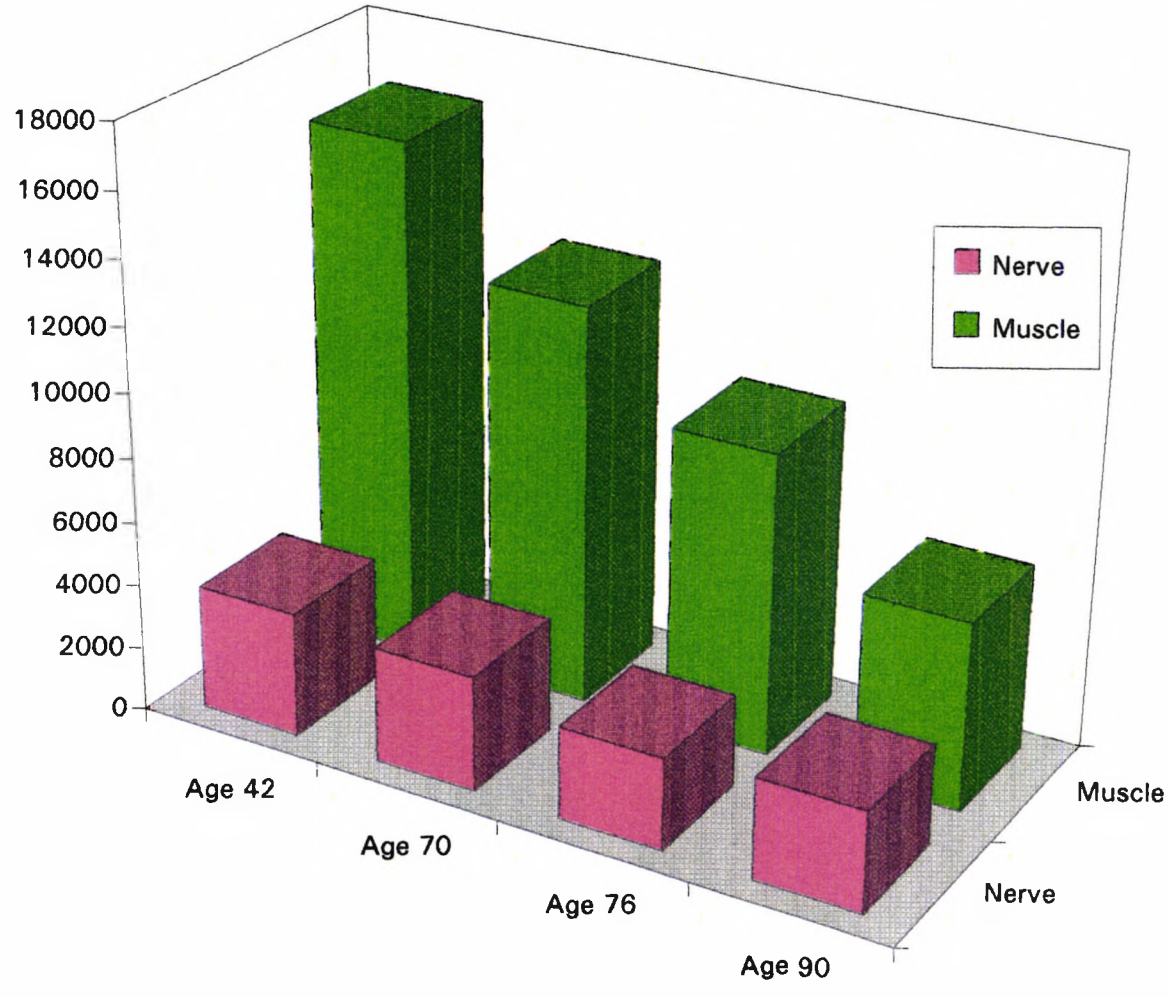


Figure 3.15 Histogram showing the nerve fibre- muscle fibre ratio in 4 inferior oblique muscles.

approach the perimysium from different directions but usually in the same longitudinal plane. This resulted in restricted areas with accumulation of motor endplates. Individual fascicles were, however, not in register with each other. The concentration of motor endplates could therefore vary considerably within the cross sectional area of the muscle.

Once inside the muscle fascicle the nerve fibres tended to run alongside their respective muscle fibres, making a few turns or even an incomplete spiral before termination. This relationship made their destination predictable several hundred microns prior to their point of termination. The motor endplates were all of the conventional type with boutons formed by the distal end of large motor axons.

Although the majority of nerve fibres terminated in the middle third region of the muscle, others of varying sizes were frequently found in both the proximal and distal regions. Nerve fibres travelling towards the distal end of the muscle were later found to terminate in tendon where they displayed nerve endings with mechanoreceptorial features (described in section 3.4). In addition to these, some smaller nerves travelled parallel with small dark staining fibres assumed to be Felderstruktur fibres.

In material obtained from two of the elderly subjects (HW11 and HW26), a few light staining muscle fibres of large diameters and well developed SR were found to be multiply innervated. Instead of terminating, these axons continued alongside the muscle fibre after the formation of a motor endplate. Several hundred microns further along the length of the muscle fibre they terminated and formed a second motor endplate (Fig. 3.16 and 3.17)

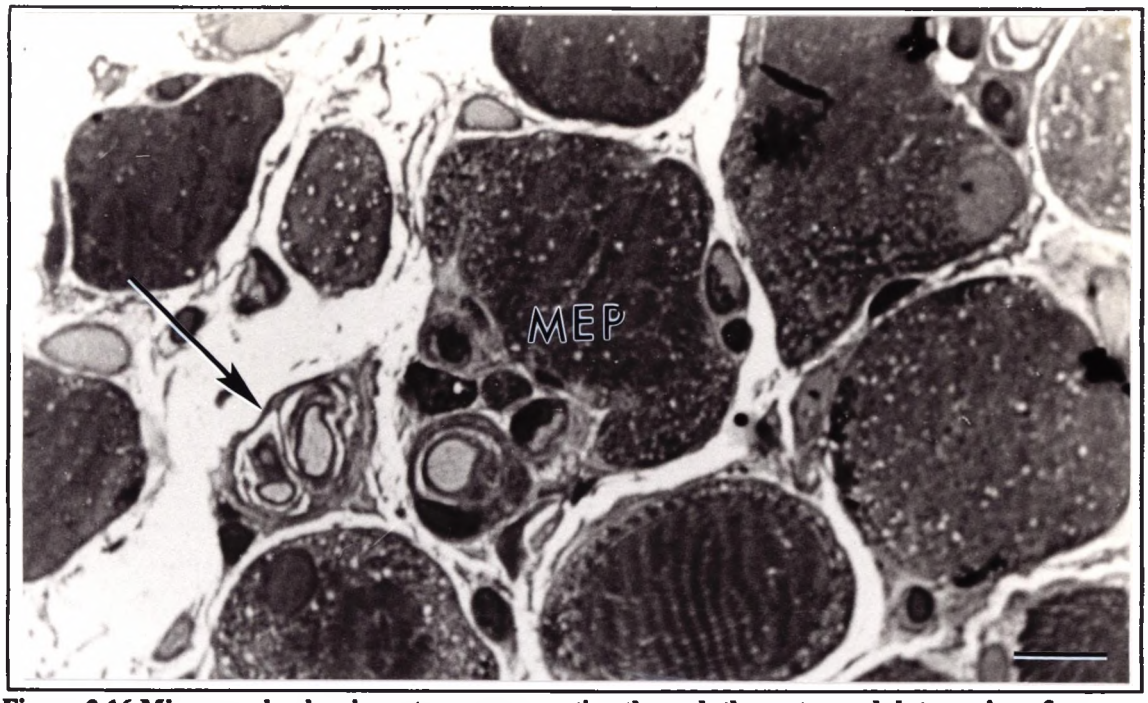


Figure 3.16 Micrographs showing a transverse section through the motor endplate region of a Fibrillenstruktur fibre. The axon (arrow) located close to the Fibrillenstruktur fibre gives off one branch which terminates on a motor endplate (MEP). The second branch continues along the muscle fibre towards a second motor endplate (evident in figure 3.17) Age of subject 90 years. Marker indicates 10μm.

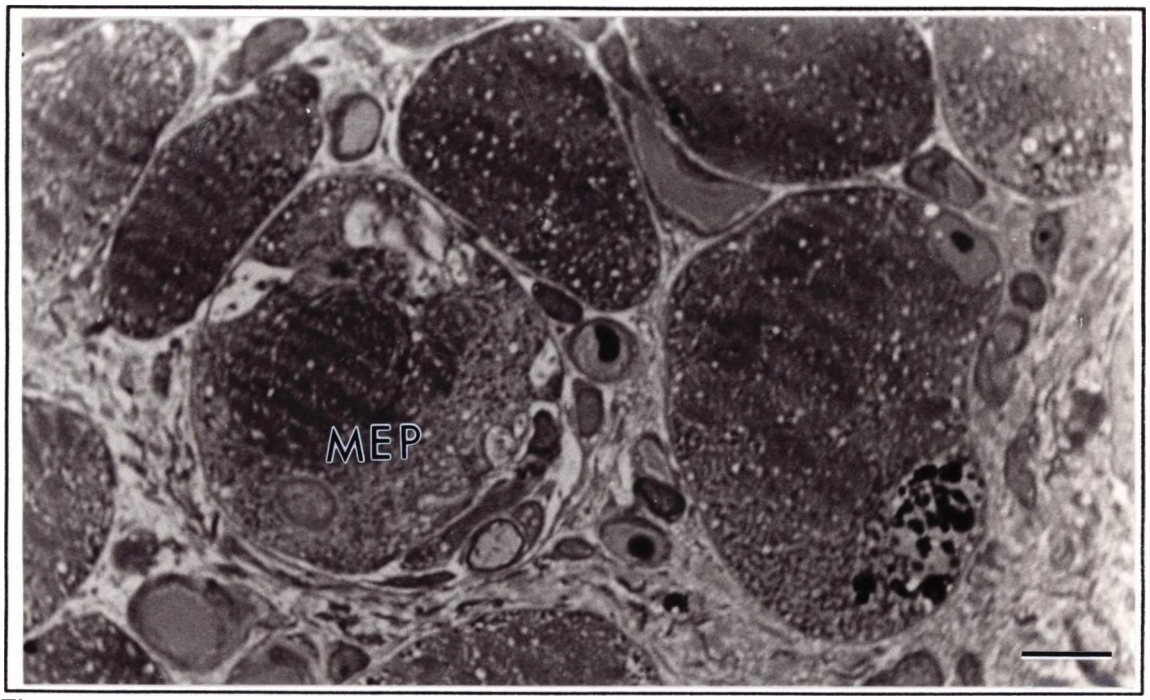


Figure 3.17 The remaining branch of the axon ran parallel with the muscle fibre for some distance before terminating on a second MEP located on the same muscle fibre. (Results from image analysis of this region enclosed in appendix G). Marker indicates 10μm.

Analysis of more than 250 efferent nerve fibres (diameter above 5μm) showed that on average they divided 2 to 3 times before they reached their destination. Due to the restricted length of the specimens the complete structural organisation of the motor units could not be found. An estimate of individual motor units without a positive identification of all the corresponding motor end plates was unsatisfactory as further axon division was possible. The difficulty in obtaining reliable results through the method described above lies in the distribution of motor endplates. The majority of all the efferent axons traced in this study had distal branches which spread over a large area of the middle third zone of the muscle. The number of sections collected in order to determine the size of a motor unit reliably was substantial because full serial sections were required. The time and effort this required was unacceptable and the method was abandoned.

In those cases where the fibres could be traced fully to the motor endplate, all muscle fibres belonging to the same unit were found to share the same morphology. These muscle fibres were traced in interrupted serial sections in search for potential polyneural innervation but no such neurological arrangement was found in this study.

3.3 MUSCLE SPINDLES

Spindles were identified in the majority of muscle samples obtained from the infant subjects. With few exceptions spindles were traced from pole to pole, and others of inadequate histological quality were not included in the analysis. Forty structures assumed to be spindles, obtained from five different muscles from 4 subjects, were studied (Table 3.1). At a later stage, on completion of the analysis 7 encapsulated structures were found to lack the principal characteristics of

spindles and these will be referred to as false spindles. These are noted but not included in the evaluation.

The spindles varied in length from 47μm to 683μm (mean 323μm). The mean equatorial diameter was 50μm. No spindles were found to have a diameter less than 10 or more than 110 μm . Even though the largest diameters were not

PATIENT	AGE AND SEX	MUSCLES	NUMBER OF PUTATIVE SPINDLES
HC 2	5 months Male	MRM IOM	17 (1 false spindle) 5 (3 false spindles)
HC 4	6 days Male	MRM	9
HC5	23 months Male	IOM	4
HC6	30 months Female	IOM	5 (3 false spindles)
SUMMARY			
4 subjects	3 males 1 female Age 6 days to 30 months	2 MRM, 3 IOM	40 spindle structures (7 false spindles)

Table 3.1 Table showing the number of spindle structures analysed (false spindles in parentheses) in various muscle samples. Information on spindle length and diameter is given in table enclosed in Appendix E.

found in the longest spindles , the results tended towards a linear relationship (correlation factor r = 0.42) between capsule length and equatorial diameter (Fig. 3.18). The periaxial space inside the spindles appeared to increase with length but there was no correlation (r = 0.07) between spindle length and muscle fibre content (Fig. 3.19.). The number of intrafusal fibres ranged from 2 to 12 (mean 5.9).

The longest spindle contained only 8 fibres while up to 10 fibres could be found in the shorter spindles, less than 200μm in length. The smallest spindles were found in the youngest subject, but due to large variations within the different age groups, a clear age related increment in size was not found.

The spindle capsule was made up of two to four laminae of fibrous cells, where several flattened nuclei could be seen in each section. A tendency to incorporate adjacent extrafusal fibres between capsular layers occurred in a majority of spindles. Embedded extrafusal fibres usually left the capsule after a short distance but some penetrated the capsule and entered the capsular space. They either terminated blindly or left the spindle pole with the intrafusal fibres.

A discrete periaxial space was not always present and there was little space around the individual fibres. Strands of connective tissue could be found connecting neighbouring fibres to each other or to the capsule wall. In a few spindles, the outline of an inner capsule could be seen but these were never

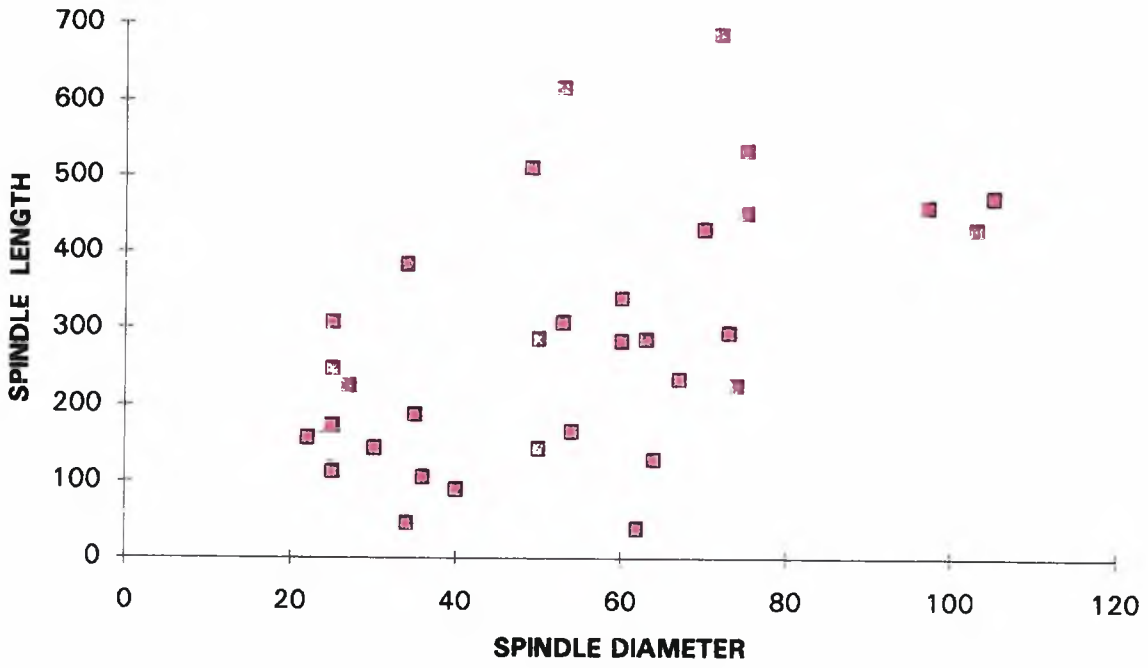


Figure 3.18 Scatter chart showing the relationship between length and diameter (correlation factor r = 0.42) of 33 spindles found in the extraocular muscles of human infants (false spindles not included.

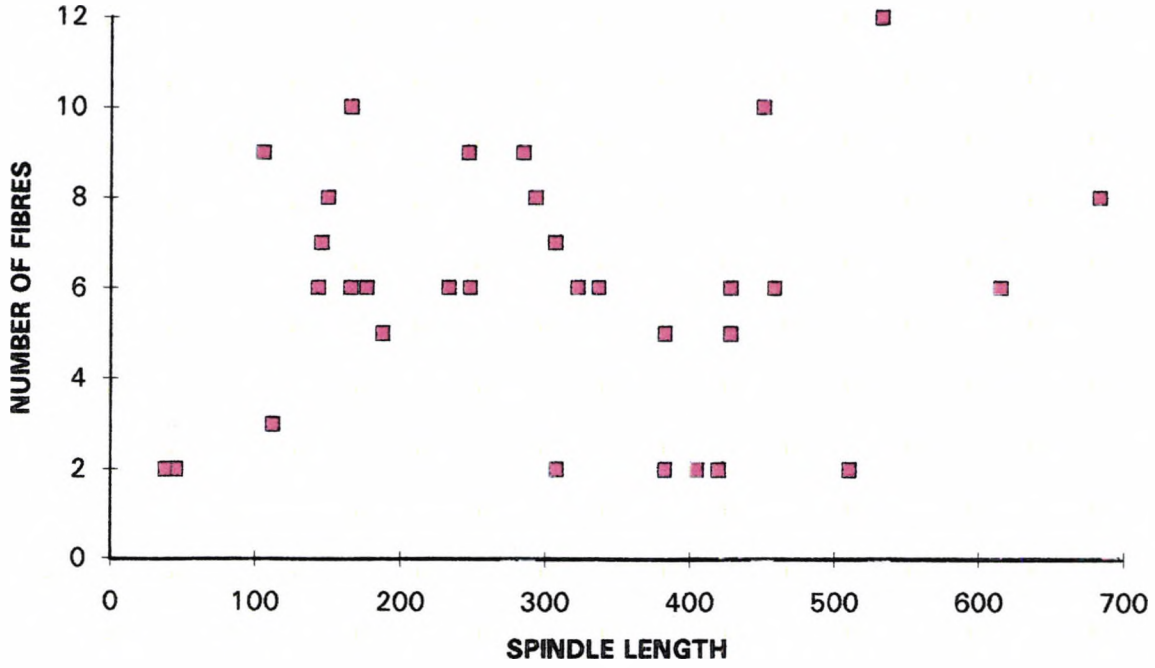


Figure 3.19 Scatter diagram showing no significant correlation between fibre content and length of spindles. False spindles not included (Correlation factor r= 0.07)

distinct or complete (Fig. 3.20) . Some spindle capsules were close to cylindrical in shape with equatorial diameters matching those of the polar regions. In these spindles the term "equatorial" had little descriptive value in the sense that no such region existed, but the term will be retained in these cases to indicate the roughly central region approximately and the region of centrally placed nuclei. Another factor contributing to the appearance of a narrow periaxial space was the size of some of the intrafusal muscle fibres. Most of them equalled or were marginally smaller than the adjacent extrafusal fibres in diameter, while the occasional fibre was bigger. On approaching the equatorial region, the expected accumulation of centrally placed nuclei, usually confined to this region of the spindle, was less distinct than in other mammals. Most centrally placed nuclei were located close to the equatorial region of the spindle, but were surprisingly also found along the whole intrafusal length of the fibre. Fibres with a nuclear content of this kind possessed little, if any, contractile elements in their polar regions. None of the spindles contained intrafusal fibres with sensory regions in register. An example of intrafusal muscle fibres with modified regions out of register is shown in figure 3.21.

Moving away from the equatorial region towards either pole, no gradually increasing internuclear distance could be found. All fibres with conventional intrafusal features were identified as being of nuclear chain type. In a few fibres two nuclei could be seen lying abreast for a distance equal to their own length, but no accumulation of nuclei or regional thickening of the fibre which could justify the term "bag" was found. As the diameter of several of the intrafusal muscle fibres was similar to that of the adjacent extrafusal fibres, their diameters varied with the location of the spindle within the muscle. Accordingly the smallest intrafusal fibres were found in spindles located in or close to the orbital region of the muscle where the majority of the extrafusal fibres were small.

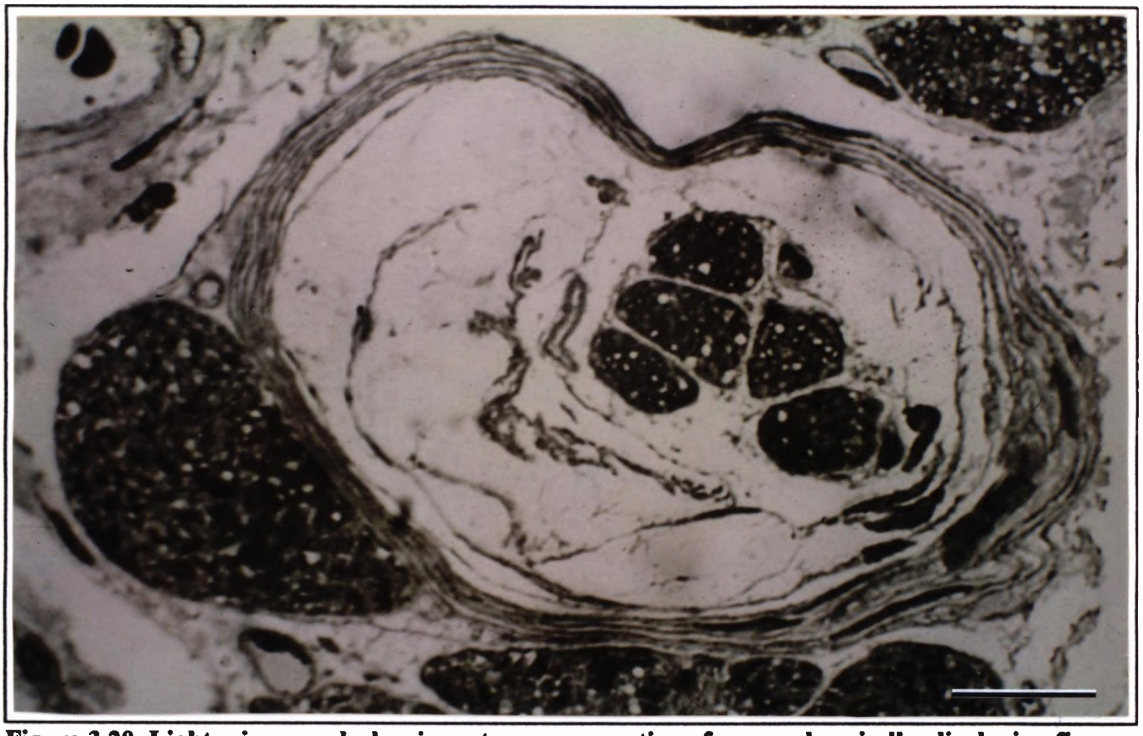


Figure 3.20 Light micrograph showing a transverse section of a muscle spindle displaying five intrafusal fibres surrounded by an incomplete inner capsule. Age of subject 30 months. Stained with toluidine blue. Marker indicates 10μm.

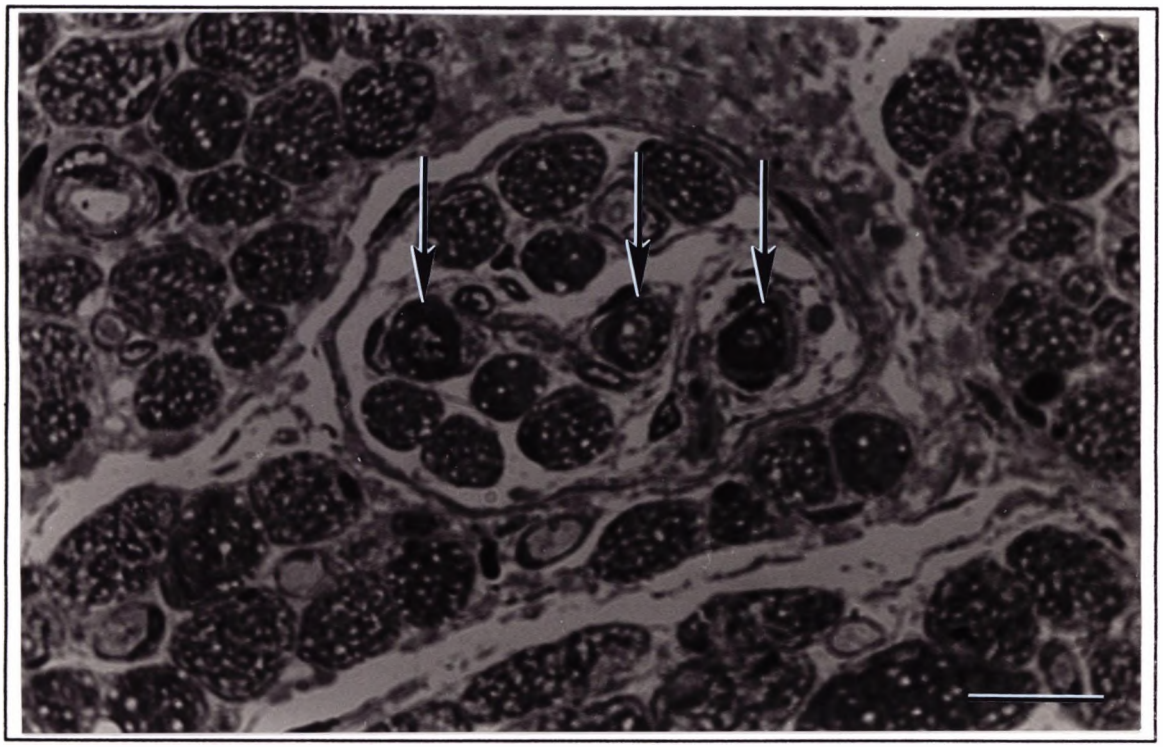


Figure 3.21 Light micrograph of a transverse section of a muscle spindle displaying 11 intrafusal fibres, 3 of them with central nuclei (arrows). Age of subject 5 months. Stained with toluidine blue. Marker indicates 10 μm.

Independent of spindle distribution, most intrafusal fibrediameters fell within the range of 5 to 25μm. However, among a total of 175 intrafusal fibres, 45(25.7%) equalled or exceeded the size of the fibres outside the capsule wall. They had peripherally placed nuclei, had no equatorial modification and no primary sensory ending was found to terminate on them. The majority of them entered the capsule at the poles along with the other intrafusal fibres. They either ran the full length of the spindle or they ran only short distances. Most commonly they ran from one pole and terminated rather abruptly without changing appearance, near the middle region of the spindle. Even in the shortest of these fibres the contractile material was continuous and only rarely fragmented.

By analysing successive sections throughout the whole length of the spindle it became clear that a large number of the remaining standard intrafusal fibres were fragmented or failed to run the full length. Consequently, the number of

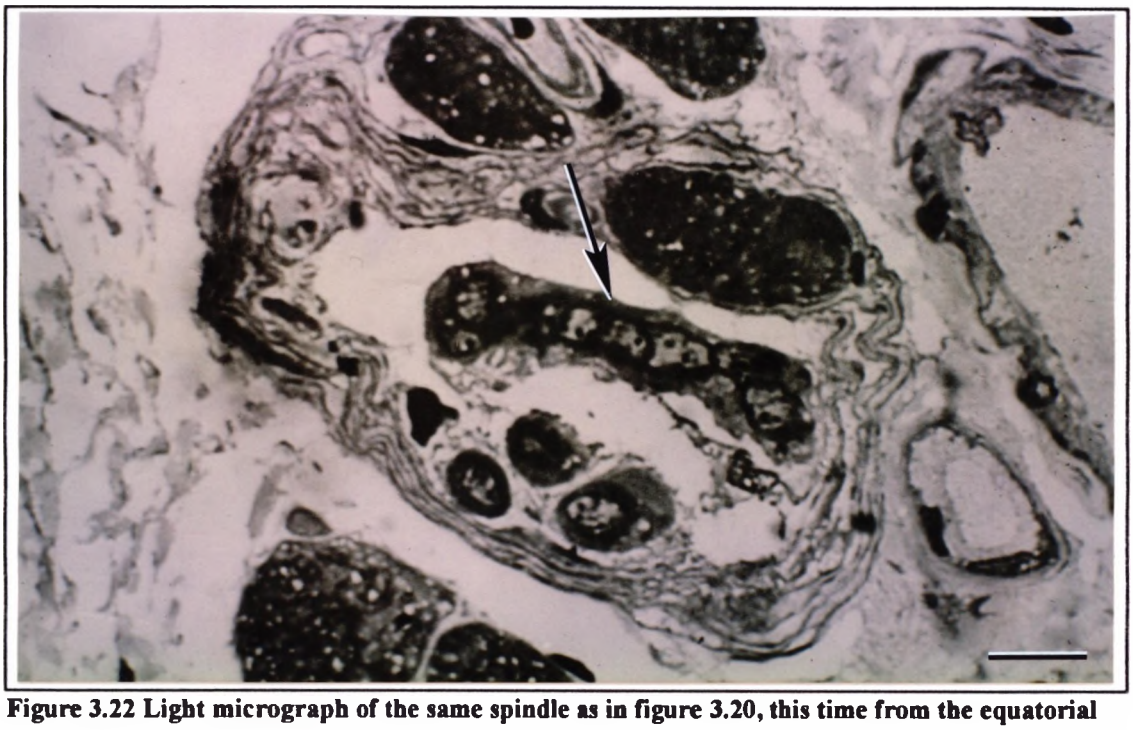


Figure 3.22 Light micrograph of the same spindle as in figure 3.20, this time from the equatorial region. The nuclear chain arrangement in the 4 fibres is best displayed in the fibre with a slightly oblique orientation (arrow). One of the five fibres in figure 3.20, terminated when approaching this region. One large extrafusal fibre is embedded in the capsule. Stained with toluidine blue Marker indicates 10μm.

intrafusal fibres varied according to the position of section. Interruptions or termination of intrafusal fibres could occur several places along the length of the spindle but most commonly at the level of the equatorial region. This was also where most of the short fibres with anomalous features terminated.

Gaps along fibres were often of substantial length (200-300μm) but small gaps down to less than 10 μm also occurred. Approaching these gaps, intrafusal fibres tapered off gradually until strands of connective tissue were substituted for the contractile component. In some cases the gap between two fibre ends was so extensive that it was not possible to establish whether they related to the same or two separate fibres. Sixty-eight (38.8%) of the fibres were fragmented or foreshortened. They were fairly evenly distributed so only 5 out of 33 spindles were free of such fibres while in 8 spindles fibres were exclusively of this type. Transverse sections obtained through the equatorial region of a few of the latter spindles were free of intrafusal fibres (Fig. 3.23).

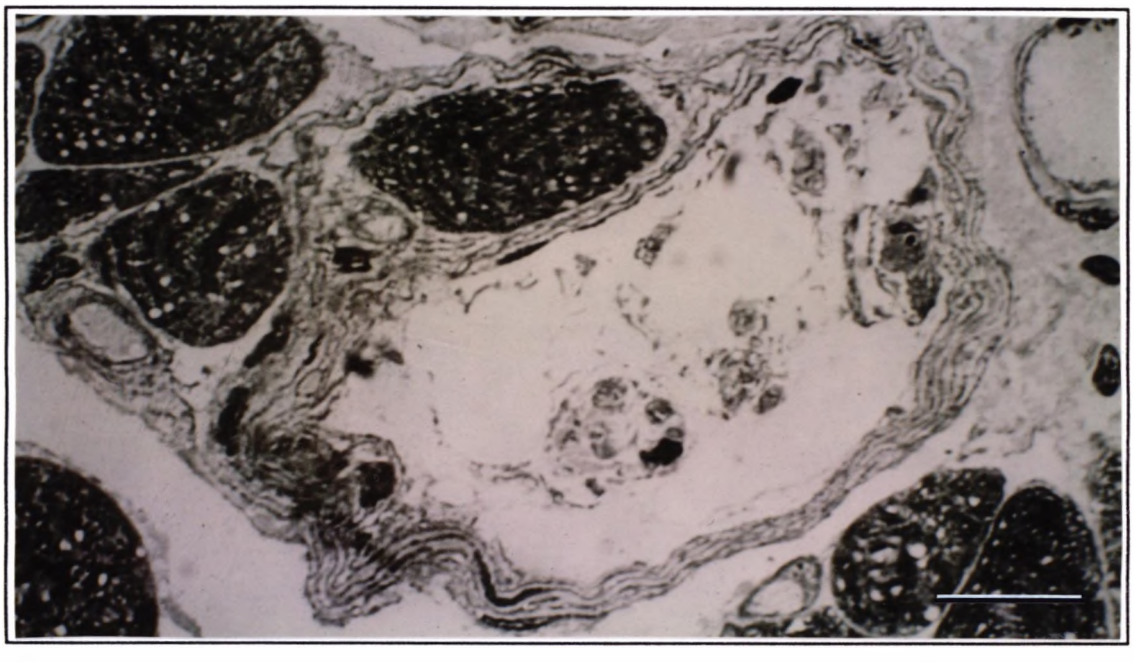


Figure 3.23 Light micrograph of the same muscle spindle as that of figure 3.20 and 3.22 In this region of the spindle, the fragmentation of the four nuclear chain fibres are in register leaving the periaxial space virtually empty. Age of subject is 30 months. Stained with toluidine blue. Marker indicates 10μm.

The remaining 62 (35.4%) fibres were identified as complete nuclear chain fibres running uninterrupted from pole to pole (Table 3.2).

The total spindle contents of muscles could not be obtained because none of the specimens included the full muscle. However, spindle counts from the middle and distal thirds of muscles revealed large individual variations. The largest

ANALYSIS OF INTRAFUSAL MUSCLE FIBRES

	NUMBER OF FIBRES	FRAGMENTED OR FORSHORTENED FIBRES	COMPLETE NUCLEAR CHAIN FIBRES
INTRAFUSAL TYPE	130 (74.3%)	68 (38.8%)	62 (35.4%)
EXTRAFUSAL TYPE (Without equatorial modification)	45 (25.7%)		
TOTAL	175		

Table 3.2 Analysis of the total number of intrafusal fibres found in 40 spindle structures.

number of spindles, fifteen, was found in the distal part of a medial rectus muscle obtained from a five months old male. A sample of the inferior oblique muscle of similar length, from the same individual, contained only five spindles.

The tendon and motor endplate regions were usually without spindles but as shown in figure 3.24, exceptions did occur. Spindles were randomly distributed transversely.

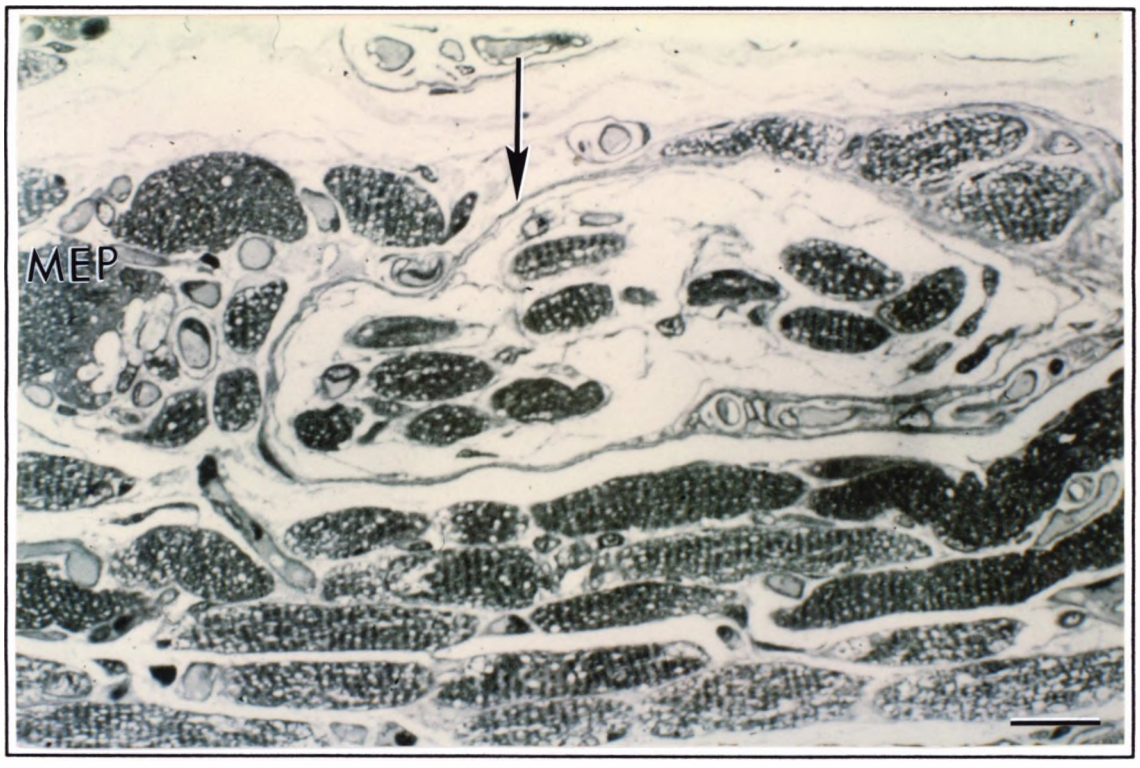


Figure 3.24 Light micrograph showing a spindle (arrow) located in the motor end plate (MEP) region of the muscle. Age of subject is 30 months. Stained with toluidine blue. Marker indicates 10μm.

The nerve fibres innervating the spindles were found to run alongside and sometimes to be embedded in the capsule wall for a substantial length before they entered the periaxial space at the equatorial region (Fig. 3.25). Blood vessels could be seen entering the capsule by accompanying the nerve fibres but could also enter through the poles along with the intrafusal fibres. Due to substantial fragmentation of the intrafusal fibres in the equatorial region, many of the sensory endings were left without a destination. In such cases, a reduction in axon diameter occurred and in successive sections the fibre tapered off and ended blindly or became indistinguishable from the strands of connective tissue in the periaxial space. The nature of the sensory endings on intrafusal fibres was hard to assess. Even though they could be found travelling alongside and sometimes in close contact with the sensory region of intrafusal fibres, they never

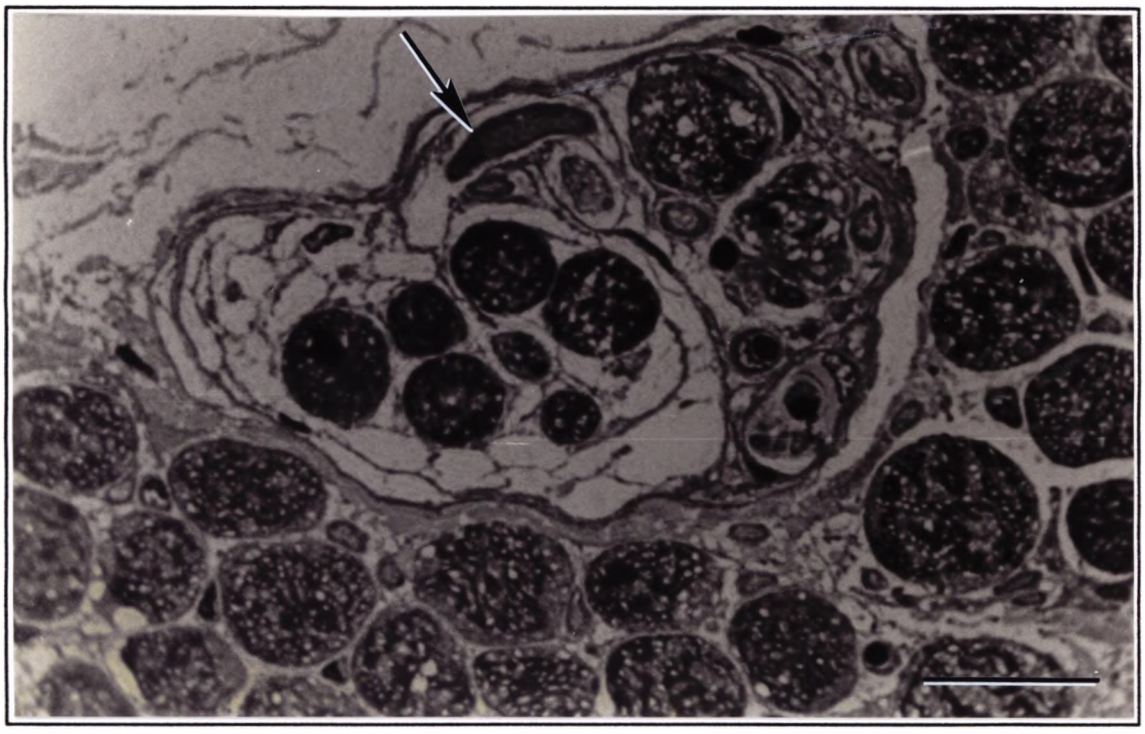


Figure 3.25 Light micrograph of transverse section through the equatorial region of a muscle spindle showing entry of the nerve fibres (arrow). Age of subject is 23 months. Stained with toluidine blue. Marker indicates 20μm.

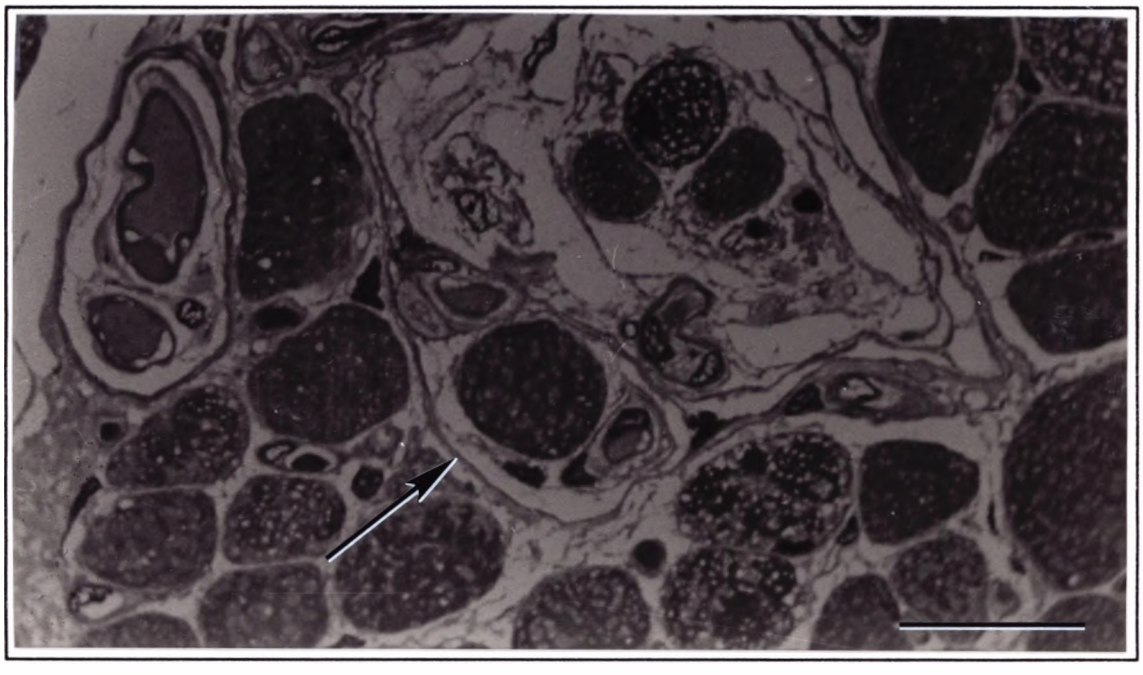


Figure 3.26 Light micrograph of transverse section through the equatorial region of the muscle spindle showing a intrafusal fibre embedded in perineural tissue of the spindle capsule (arrow). Age of subject is 23 months. Stained with toluidine blue. Marker indicates 20μm.

displayed distinct spirals. Myelinated and with a size ranging from 5 to 15 μm, the sensory nerve fibres were indistinguishable from the motor fibres. Their site of termination was the strongest indicator of their afferent nature.

Among the structures, initially identified as spindles, there were groups of encapsulated extrafusal fibres. These fibres did not show any modification in size or nuclear distribution, nor did they have fibre modifications. They equalled the extrafusal fibres in size and the capsule invested these fibres tightly throughout the encapsulated length. The capsule was made up of the endo- or perineurium of the neighbouring nerve(s). Most of these structures were found close to the motor endplate region or other nerve rich regions (Fig. 3.27). Nerve fibres which could be seen running alongside these structures never entered the capsule. Single encapsulated fibres were also present, but these structures were mostly associated with motor endings encapsulated by the perineural sheet.

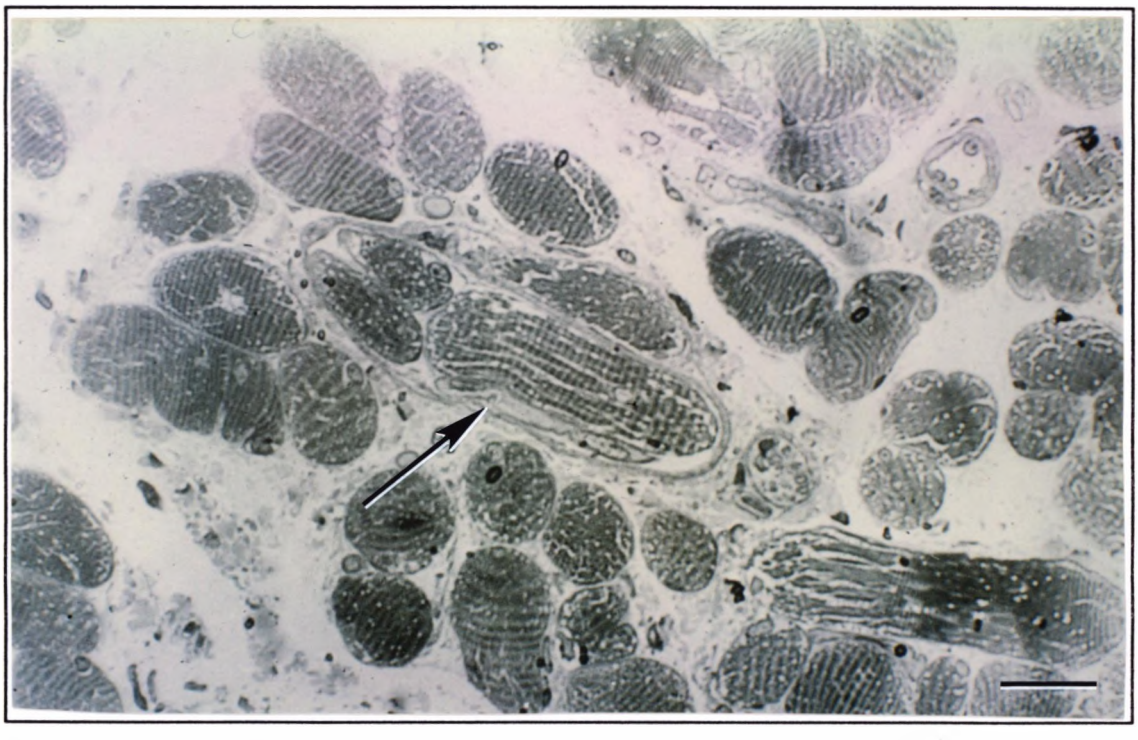


Figure 3.27 Light micrograph showing encapsulated extrafusal muscle fibres. False spindle (arrow). Nerve fibres (arrowhead). Age of subject 5 months. Stained with toluidine blue. Marker indicates 10μm.

3.4 TENDON RECEPTORS

No axons were found in the distal region of the muscles obtained from infant subjects. Occasionally nerve fibres either associated with muscle spindles or bloodvessels (Fig. 3.28) were found in the distal thirds of the muscle but none were found in or approaching the myotendinous region. Efferent axons were also observed but they terminated exclusively on Felderstruktur fibres (Fig. 3.29). The distal ends of all the latter type of fibres terminated before they reached the myotendinous transition zone. This result was not anticipated and particular care was given to the examination of all the infant specimens before concluding that none contained nerve fibres. The absence of neural elements in the distal end of infant specimens (including small unmyelinated nerve fibres) was confirmed by electron microscopical inspection.

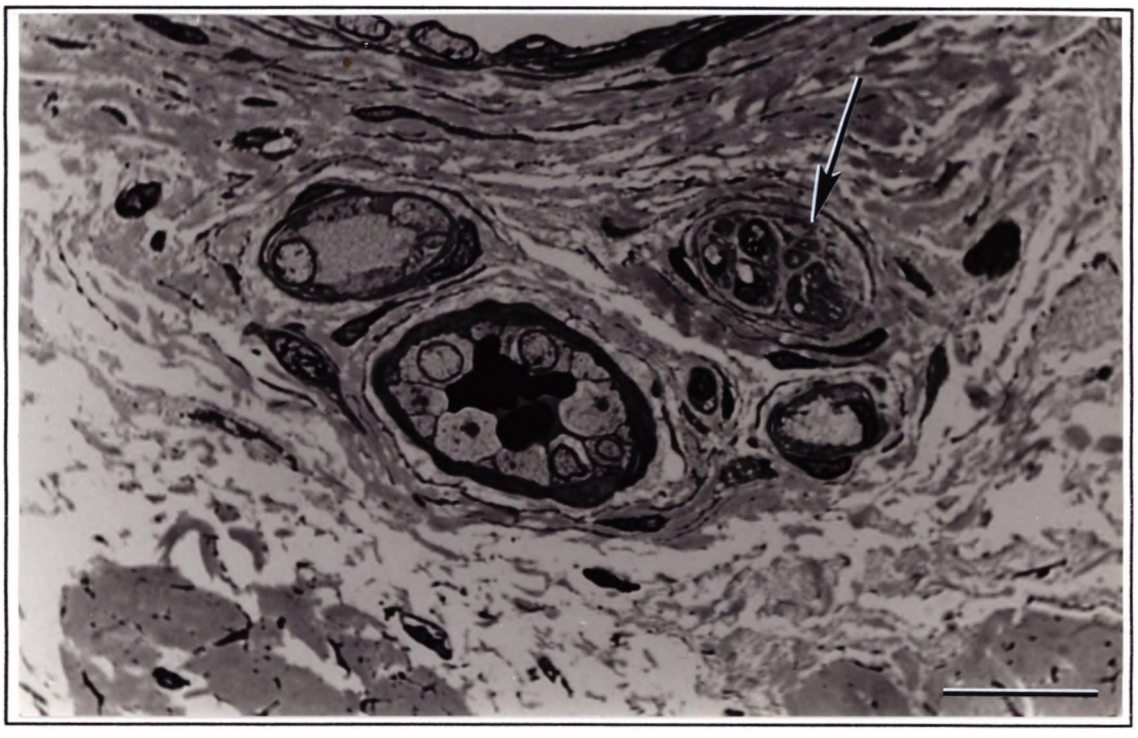


Figure 3.28 Light micrograph of transverse section through the myotendinous region showing small nerve fibres closely associated with blood vessels (arrow). Sample obtained from a 5 months old subject. Stained with toluidine blue. Marker indicates 20 μm.

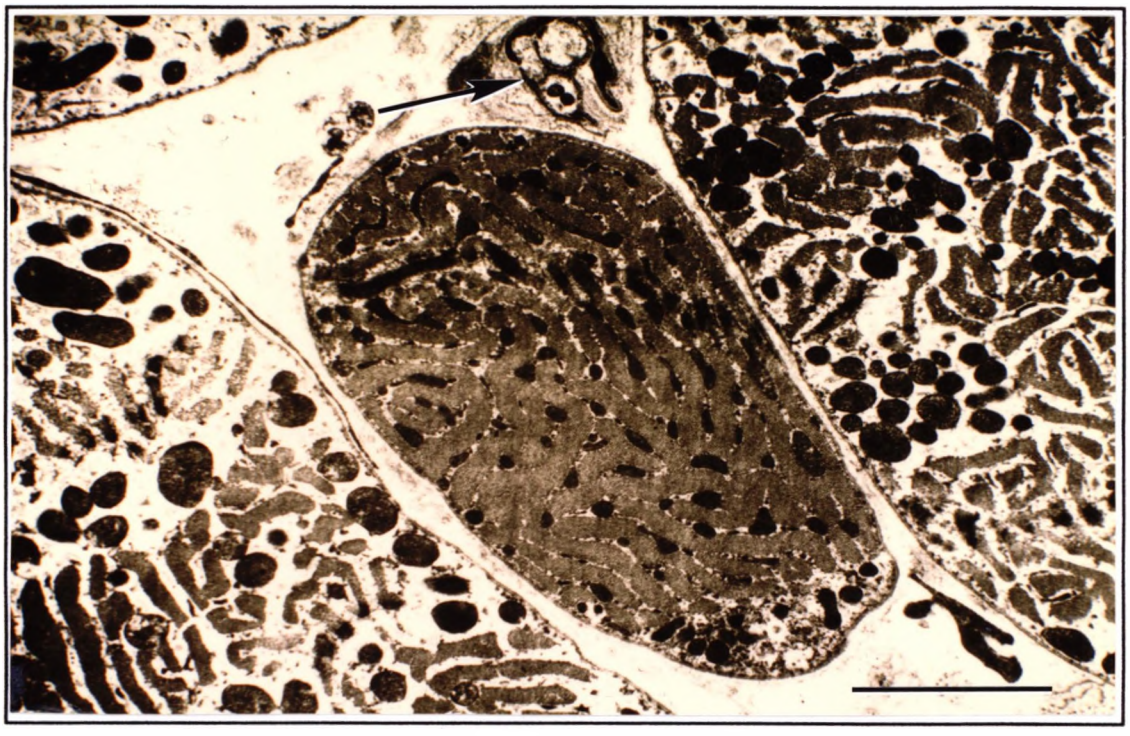


Figure 3.29 Electron micrograph of Felderstruktur fibre in transverse section of infant subject (5 months). Note the small diameter efferent pre terminal nerve fibre (arrow). Marker indicates 5 μm.

There were individual variations in the length of the musculotendinous transition zone as well as variation according to the particular muscle and age of subject. But in most cases the first muscle fibre termination occurred peripherally, marking the beginning of the myotendinous interface. The proximal part of the tendon therefore lay marginally, forming an incomplete surround to the muscle (Fig. 3.30).

In samples from mature subjects (21 to 74 years), unmyelinated and myelinated nerve fibres with diameters ranging from 1 to 6 μm were frequently observed throughout the length of the myotendinous junction. They ran parallel with the muscle fibres for a substantial length and terminal boutons were occasionally found at the myotendinous junction . These distally located nerve terminals were not exclusively associated with Felderstruktur fibres; some of them were

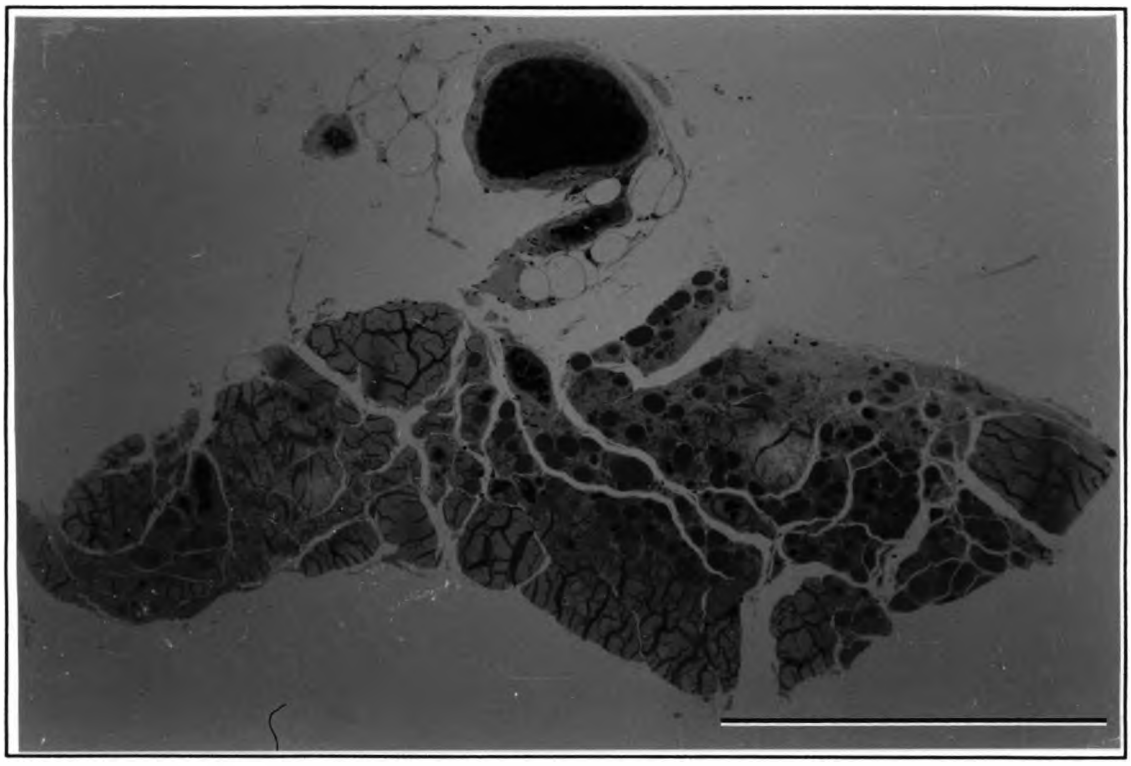


Figure 3.30 Light micrograph of a myotendinous region in transverse section. Superior rectus muscle obtained from an adult subject (74 years). Stained with toluidine blue. Marker indicates 500μm.

also in the form of conventional motor end plates (Fig. 3.31). Some nerve fibres extended even further to the inserting tip of single muscle fibres. In this region the muscle fibres split into several processes which in transverse sections appeared as circular profiles with a myofilamentous content. The myofilamentous material could be seen as densely punctate material separated from the surrounding collagen by a seemingly intact sarcolemma (Fig. 3.32). The diameters of these areas gradually decreased and then terminated in collagen, often with numerous cells with flattened nuclei assumed to be fibroblasts. Most muscle fibres terminated in this fashion.

The largest of the nerve fibres associated with the muscle processes had a thin ring of myelin which was lost as the axon approached the musculotendinous junction. In most cases the axons were subsequently traced to a position between the terminal processes of the muscle fibres. At this level all fibres were

unmyelinated and judging by the regular occurrence of dark staining nuclei they were enveloped by a single Schwann cell. There were regions where the separation between the contractile elements of the muscle fibre and the axons was minimal. Despite this seemingly close relationship, axons were not found to terminate directly on the muscle processes. Small isolated axon profiles were occasionally observed close to the traced fibre giving the impression that they represented branches of it.

These neural arrangements were observed associated with terminal processes of muscle fibres of various diameters. But in several cases they were also found to terminate in tendon remote from muscle fibres, either prior to the muscle fibre termination or further into the tendon region.

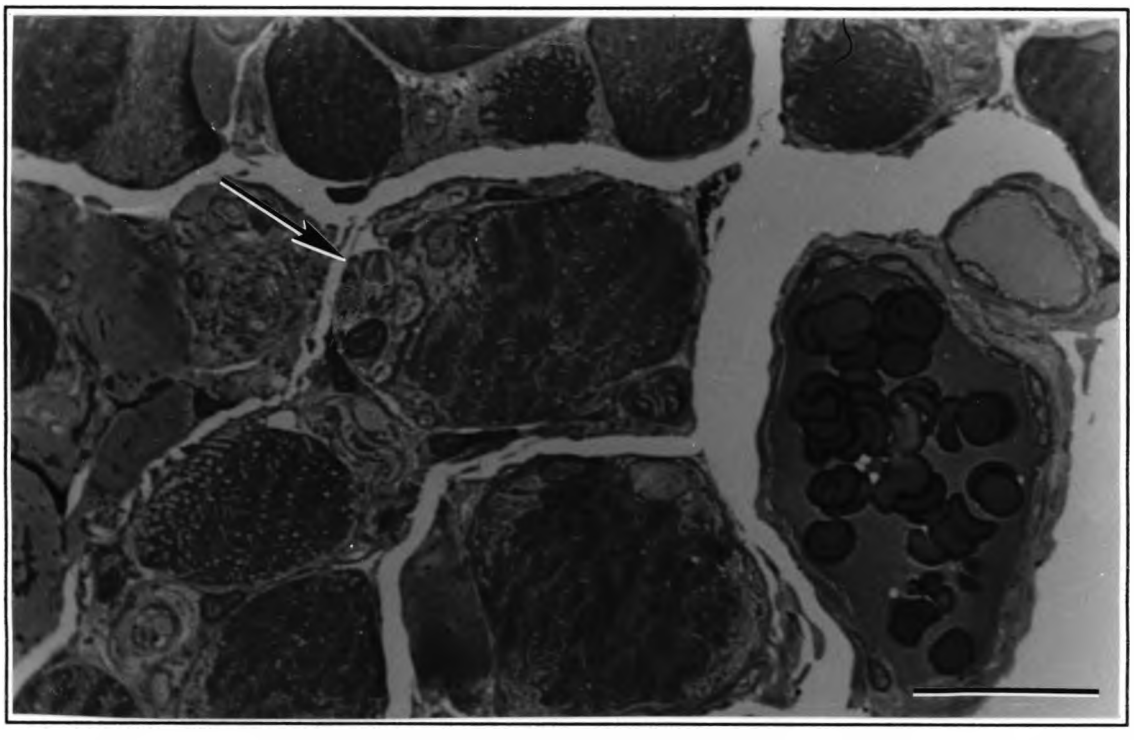


Figure 3.31 Light micrograph of a Fibrillenstruktur fibre in transverse section through a motor end plate (arrow). The abundance of tendon shows the distal location of this neuromuscular junction. Obtained from an 74 year old subject. Stained with toluidine blue. Marker indicates 20 μm.

Hence, the difference between the myotendinous regions of infant and mature muscles was profound.

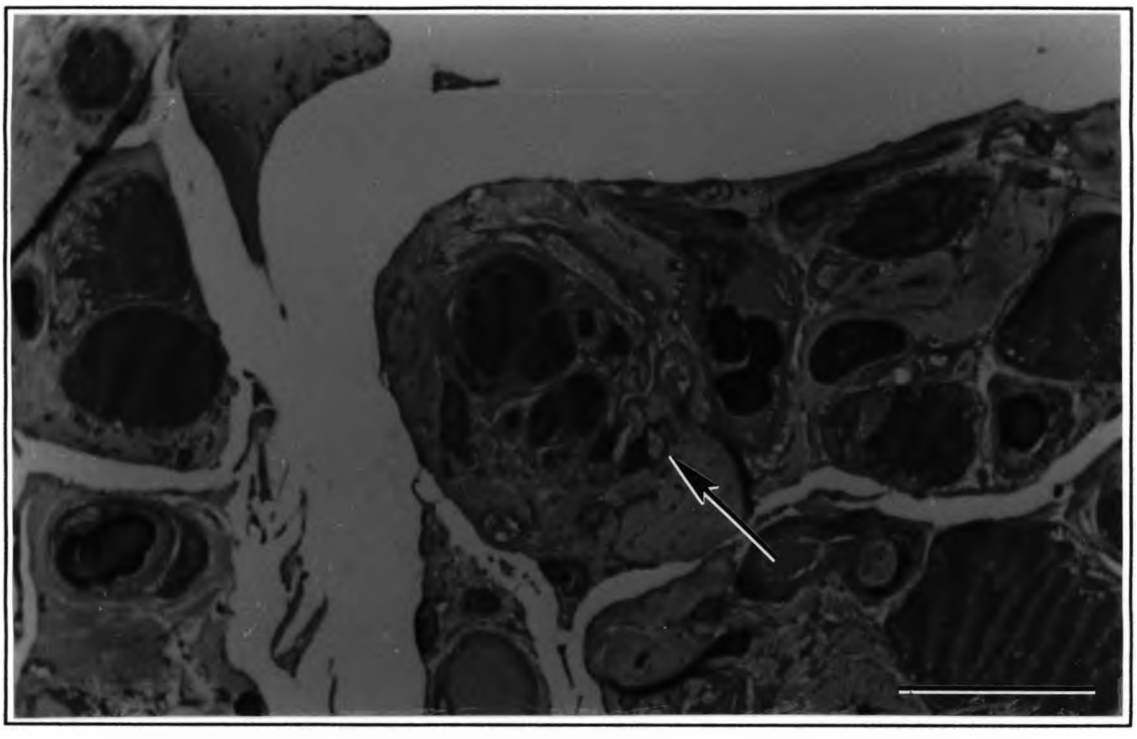


Figure 3.32 Light micrograph showing processes of the distal end of the muscle fibre. The axon (arrow) runs close to these processes but does not terminate directly on them. Obtained from an 74 year old subject. Stained with toluidine blue. Marker indicates 20 μm.

CHAPTER 4

DISCUSSION

4.1 GENERAL MORPHOLOGY

Observations of the material put to use in this study indicate that the extraocular muscles of man go through various morphological changes during life. In view of the relationship between structure and function, it is reasonable to assume that structural changes brought about by growth or maturation affects the muscle's contractile properties. In the infant material there are numerous muscle fibres with immature features. The presence of successive centrally placed nuclei with features matching the myotube cell of immaturity is suggestive of post parturitional development.

Subsequent to maturity, structural changes occur which cannot be considered beneficial to the contractile mechanism of the muscle. In the Ringbinden fibres, the orientation of the peripheral myofibrils suggests that they contract along an axis perpendicular to the length of the muscle fibre. None of the specimens from infant subjects contained such structures and they were first observed in a 45 year old subject. This is consistent with previous studies, where a substantial increase in Ringbinden fibres was found to occur with increasing age ,especially after the age of 60 (Mühlendyck and Ali, 1978). Presumably the constant mechanical stress of myofibril contraction eventually leads to disruption and atrophy.
Even though the incidence of Ringbinden fibres has been found to be no more than a few hundred fibres (538 reported in a 83 year old patient) it has been suggested that these structures may be responsible for muscle dysfunction in older individuals (Mühlendyck and Ali, 1978) . In the current study identification of fragmentation and loss of myofilaments unrelated to the Ringbinden fibres would contribute to such dysfunction. Conclusive evidence as to at what stage in

life these changes first occur could not be provided from the material used in this study. These observations are, however, consistent with previous studies where indications of histological changes have been found in all EOM with advancing age. More detailed studies of these diffuse changes (Miller, 1975), have led to the contention that they may well account for alterations of eye movements observed in the aged. The concept of structural age related changes in the muscle fibre population during life is attractive in the sense that it must have some functional implications. The extent of these changes, however, remains uncertain since quantitative analysis of the fibre population in human EOM of various age groups is not available.

The difference in the number of muscle fibres between the youngest and oldest of 4 subjects, in this study was substantial (63.6%) with a clear correlation between age and fibre content (r = 0969). Because of the small number of subjects these results cannot be considered as tangible evidence for a reduction in the fibre population with age. They do, however, encourage further study in view of the functional implications such a fibre reduction would have were these figures to be representative for human EOM in general. Such a reduction in the number of muscle fibres would play a vital role in the gradually diminishing ocular motility which has been pointed out in various functional studies (Holland,1956: Chamberlain, 1971). If the alleged fixed relationship between efferent activity and eye position is to be maintained throughout life it must undergo adjustment as muscle force capacity changes.

4.2 MOTOR UNITS

A bimodal distribution of myelinated fibres in human oculomotor nerves was found by others with peaks corresponding well with the ones obtained in this study (Torre, 1953). A further peak is now added by including unmyelinated fibres in the count.

In the current study, unmyelinated or myelinated fibres with a diameter less than 4μm were never observed to terminate on Fibrillenstruktur fibres. Likewise, large myelinated fibres were never observed terminating on Felderstruktur fibres. Hence, the bulk of fibres with small diameters innervate the Felderstruktur fibres. Similar findings have been reported by others (Mühlendyck and Ali, 1978). In consideration of the motor unit it is of some value to distinguish between Felder and Fibrillenstruktur fibres, bearing in mind the limited contribution the Felderstruktur fibres make to muscle contraction.

4.2.1 The Motor Unit for Felderstruktur Fibres

A mean of the number of nerve fibres, 4μm or less in diameter from the nerves of all IOM preparations in histogram 3.7, gives a figure of 1428 . The mean value for the content of muscle fibres in the 4 muscles was 10966. Apart from a single report (Namba et al.,1984) there is general agreement of the quantity of Felderstruktur fibres. They constitute roughly 20 % of the total in the human EOM (Mühlendyck and Ali, 1978 and Ringel et al., 1978). It follows from this figure that unless the number of sympathetic fibres and fibres from various receptive end organs is very high , the motor unit of the Felderstruktur system must be very small. Using the 20% factor, 2193 muscle fibres are served by 1428 nerve fibres (ratio $\supseteq$ 1.5). This ratio is applicable on the assumption that polyneural innervation is not present.

Estimates of the motor unit have been criticised because the sensory and autonomic fractions of the nerve fibres have not been taken into account. Quantitative studies of the sympathetic nerve supply to human EOM is not available but superior cervical ganglionectomy in monkeys has shown that these nerves constitute up to 30% of the unmyelinated nerve supply to the IOM

(Ruskell, 1983). If we assume that a similar figure is true for man and this proportion is subtracted from the small nerve fibre total, the unit is still small (2.1, 2193 : 999).

4.2.2 The Motor Unit for Fibrillenstruktur fibres

The required allowance for nerves serving sensory receptors may not be large, as suggested by the results of this study. Consistent with previous reports (Ruskell,1983) structures described by others as sensory (Cooper and Daniel, 1945) were found to have distinct efferent features. It is furthermore reasonable to assume that the presence of false spindles, first described by Ruskell and verified in this study, have led to overestimations in previous reports on spindle content (Cooper and Daniel, 1945; Merrillees et al,1950). This view was recently confirmed in a detailed study on the number and distribution of spindles in human EOM (Lucas et al, 1994). In agreement with results from this study these workers found fewest spindles (ranging from 3 to 7) in the IOMs. These receptors contained an average of 5 intrafusal muscle fibres, each connected to an afferent axon. The tendon receptors which receive only one single nerve fibre were found to be even less numerous. In all the muscles examined the number of nerve fibres terminating in the distal end of the muscle never exceeded 1% of the total. After accounting for these sensory nerves there remained a total of 1857 nerve fibres with a diameter of 4 μm. or above. On the assumption that 20% of the muscle fibres in EOM are of the Felderstruktur type, the remaining 80% (in this case 8772) are of the Fibrillenstruktur type. Calculations of the motor unit for the Fibrillestruktur fibre system resulted in a clearly higher ratio than the one obtained for the Felderstruktur system. A total of 8772 muscle fibres was served by 1857 nerve fibres ≈ 4.7.

It seems likely that the differences in the neuromuscular arrangements of the Felderstruktur and Fibrillestruktur have functional implications. Accepting the relationship between motor units and motor control, the muscle fibres controlled by the smallest units would have responsibility for delicate movements and the tuning of these. It is attractive to interpret the current data as support of such a role for the Felderstruktur fibre. In contrast to the Fibrillenstruktur system, recruitment of single Felderstruktur fibres could theoretically occur, resulting in very small additional forces giving the basis for fine gradations in muscle contraction. Our present knowledge of the physiology of these fibres is incomplete. Further comment on the role of these fibres would be speculative and therefore will not be attempted.

The ratio of total muscle and nerve fibres in the present study is low (mean 3.33) compared with previous studies. Bors (1926) found a ratio of 7 in the IOM of man while similar studies on the SOM gave ratios of 5.3 and 6.8 (Torre, 1953). Although Torre did not include any photomicrographs in his paper, his use of thick paraffin sections suggests a resolution no superior to the results obtained by others using the same method (Tergast,1873; Bors ,1926; Goldschmidt,1969). It is highly unlikely that the full population of myelinated axons was included in these previous analyses. The unmyelinated nerve fibres which constitute up to 20% of the total , have a diameter barely within light microscopic resolution and must have been overlooked in the thick sections used. Furthermore, Bors' data on the IOM was based on one single observation and offers no information on individual variations.

Increments in the muscle fibre population of human EOM have been observed from infant to young adult life (Kato, 1938;Goldschmidt, 1969). But the material used in each of these studies consisted of only two subjects and in both studies there was a certain inconsistency in the fibre increment between the

various muscles. Neither of these studies refers to the presence of immature muscle cells as found here. Information on changes in the fibre population in human EOM from mid life to old age is not available.

Loss of functioning motor units in old age has been reported in somatic musculature and the notion that this process commences after the age of 60 have been supported both electrophysiologically (McComas ,1991) and anatomically (Tomlinson et al,1977). This loss is caused primarily by gradual degeneration of efferent nerve fibres. A similar loss in the oculomotor neuromuscular system might be expected. Results obtained in this study do not support such a change. The fibre populations in 7 nerves did vary with age but the modest decrement between the youngest and oldest subject (1.6 %) along with individual variability does not offer support for an age deficit. However, the size of the motor unit decreases proportionally, with an apparent age related reduction in muscle fibres. The large range from 4.74 (found in the youngest patient) to 1.87 (found in the oldest patient) is due to the fact that the latter specimen contains 10277 fewer muscle fibres than the former. The view that this represents no more than individual variations cannot be dismissed but seems very unlikely in view of the identification of histological changes, mentioned above, indicative of degeneration with age.

4.3 MUSCLE SPINDLES

Muscle spindles in the extraocular muscles of human infants share the anomalous features previously recorded in adults (Ruskell,1989). The argument for questioning a proprioceptive role for these receptors applies therefore equally for young and old muscles. In Cooper and Daniel's study (1949) the muscle samples were taken from a wide range of ages including infant material. While most of the anomalous receptor features were overlooked by these workers, the

presence of a narrow periaxial space with large intrafusal fibres was described. A comparison between young and old material was not recorded, but photomicrographs gave little evidence of differences between the two. Cooper and Daniel's observations are consistent with the findings in this study.

It is generally accepted that the function of a fluid filled space is to protect the intrafusal fibres by dampening the effect of the extrafusal fibres during a muscle contraction (Brzezinski,1961). This facility is effectively denied to EOM spindles. Due to a restricted equatorial diameter, the human extraocular spindles appear crowded , leaving little distance between the individual intrafusal fibres as well as to the surrounding capsule. This factor argues against the spindle's ability to function as an isolated unit free from interference by extrafusal activity. The impression of interference with spindle function is further enhanced by the presence of large anomalous intrafusal fibres free of equatorial nucleation. These fibres were present in the majority of spindles as previously observed in mature muscles (Ruskell, 1989). Their functional state will presumably influence spindle length and disturb the response of spindle afferents.

Anomalous intrafusal fibres were present in the majority of spindles and as many as 8 were found in one spindle. They were first observed in human adult material and suggested to be expressions of spindle reorganisation due to degeneration (Ruskell,1989). EOM spindles are the receptors thought to provide the proprioception which has been claimed be so vital for the development of binocular vision in other animals (Hein and Diamond,1983). This view could be sustained in man despite a redundancy of proprioceptive end organs provided the changes were associated with ageing. But the fact that anomalous intrafusal fibres are present in both adults and infant specimens is, however, inconsistent with such a view. The same argument can be applied when discussing the false

spindles. These structures were suggested to represent the culmination of spindle degeneration with all bag and chain fibres replaced by erstwhile extrafusal fibres (Ruskell, 1989). This is not consistent with their presence in infant muscle tissue.

The richness of spindles has been commented by many authors and is a favoured argument for the fine oculomotor control. The present findings challenge this concept. The identification of false spindles points to an overestimation of the number of spindles in the earlier studies. This view is consistent with the relatively modest number of spindles reported by Lucas and co-workers (1994) after deduction of structures identified as false spindles. The regular distribution of spindles found by these workers could not be confirmed in the current study due to the restricted length of muscle samples. Regarding the true spindles, their viability as providers of proprioceptive information is questionable. The fragmented intrafusal nuclear chain fibres are arguably the ultimate factor which jeopardises a potential normal spindle function. It seems highly unlikely that any deformation of the primary sensory ending would occur in these fibres during a muscle contraction. Taking into account these and the other factors described, it seems that the spindles in human extraocular muscles have phylogenetically lost their proprioceptive capacity.

The delay in the fixation of the infant material resulted in certain histological imperfections at light microscopic level. These shortcoming were mostly observed in the axoplasm and found to be consistent with the notion that nervous tissue displays changes earlier in the temporal sequence of autolysis than most tissues. The view that the anomalous organisation of intrafusal fibres observed in this study should represent artefacts induced by autolysis seems unlikely. The presence of anomalous intrafusal muscle fibres in sections containing nerve fibres with intact axoplasm supports this notion.

4.4 TENDON RECEPTORS

If muscle spindles are incapable of providing proprioceptive information there remains only one other acknowledged muscle related source- the Golgi tendon organ or its equivalent. Typical Golgi tendon organs have been found in the EOM of several domestic animals but not in man (Hunt,1974). Their absence does not preclude the presence of other receptors fulfilling a similar role.

Studies of the distal neuromuscular junction of man have revealed various neuromuscular complexes with mechanoreceptorial features (Richmond et al, 1984). Their morphology departs from that of the Golgi receptor in that they lack the conventional fluid-filled capsule. Furthermore, rather than the numerous muscle fibres relating to the typical Golgi receptor, muscle and nerve fibre have a one to one ratio.

Results from the current study are largely consistent with these previous observation. There were however a few differences. Although delicate, both the palisade ending and the musculo-tendinous complexes of Sodi et al (1988) have an envelope of connective tissue. The presence of such an investment is claimed to be a feature which strengthens the potential sensory role of these structures (Alvarado-Mallart & Pincon-Raymond, 1979). Furthermore, the neural element was observed to terminate on the processes of the muscle fibre tip or in invaginations of the sarcolemma prior to the musculotendinous junction. Nerve terminals with such locations could easily be compressed during muscle contraction or by passive stretch and hence act as force transducers providing afferent feedback. Association between nerve terminals and the distal tendon in adults is also described in the present study but in certain details the results differ from those cited. For example, an intimate relationship between muscle and nerve was not observed; although closely associated , the axons did not terminate directly on myofilamentous structures. Nor was a surrounding envelope present. The fragments of connective tissue observed surrounding some of these

complexes did not constitute a capsule. However, even in the most simple form a neurotendinous arrangement constitutes a potential mechanoreceptor. The neural elements presented an appropriate disposition for playing a proprioceptive role and were arguably close enough to be influenced by the mechanical force transmitted through contraction of the muscle or by passive stretch. Tensile forces exerted on the collagen in which the nerve fibre lies could easily lead to deformation of the membrane, followed by changes in permeability.

The possibility of an effective proprioceptive role for the nerve endings in mature myotendon diminishes in view of their absence in infant tissue. The frequent occurrence of musculotendinous regions free of innervation in man have been commented on by others (Richmond et al 1984). But the notion that all regions containing receptors should have been missed in the current study due to such irregular distribution seems highly unlikely. The post mortem delays resulted in some autolysis but most of the neural elements were preserved in good condition. Despite adequate histological quality , exhaustive attempts did not unfold any neural presence in the tendon region of material obtained from subjects up to 47 months of age. The disparity between infant and mature tissue clearly indicates that myotendinous nerve fibres appear with age. This observation is inconsistent with previous views where muscle proprioception is thought to be an important factor in the development of various binocular functions (Bridgeman & Stark,1991). The absence of tendon receptors and a questionable availability of muscle spindle function dismisses the veracity of this argument.

The presence of nerve endings in the distal end of Felderstruktur as well as Fibrillenstruktur fibres in the mature muscle suggests a capacity to monitor activity of both the slow and fast contracting system. But although the neural arrangement in both types of muscle fibres appears similar, one cannot be

certain of the sensory nature of all the nerves terminating on the distal end of these fibres, especially those terminating prior to the formation of the muscular processes. Consistent with findings in somatic muscles, eye muscles undergo progressive degeneration throughout life (Miller, 1975). In somatic muscles in rat, such changes have recently been found to include remodelling of motor nerve terminals (Larsson and Ansved, 1995). In the current study, some of the Fibrillenstruktur fibres were found to have a peculiar efferent innervation (section 3,2) in the form of more than one motor end plate. Furthermore, motor end plates, which are usually confined to the middle third region of the muscle were occasionally found in the distal end. This was found to be a feature exclusive to mature muscles and might therefore be an expression of motor endplate lability. If this is tenable it may account for some of the tendon fibres, arguably those which could not be traced the full length to the myotendinous transition zone. These fibres represented a clear minority.

The view that afferent innervation is subjected to a similar lability cannot be dismissed. Since the myotendonous region in the infant muscles was presumably without innervation there is a possibility that the potential sensory fibres found in the mature muscles are also accounted for by displacement. In this instance it is tempting to suggest that they occur randomly as a result of sensory fibres made redundant by the breakdown of spindles.

4.5 OTHER SOURCES OF PROPRIOCEPTION

The possibility of proprioceptive sources other than muscle cannot be dismissed. Among the sensory receptors in the anterior aspect of the eye there are structures with mechanoreceptorial features (Lawrensen & Ruskell,1991). Due to their disposition in relation to the oculorotary elements, these receptors would only provide feedback regarding eye movements, not muscle activity. The fairly

constant location of the eyelid with respect to the globe could presents a reference point for monitoring relative eye movements. These considerations can only be speculative and there is no reliable information to suggest a capacity to monitor the position of the eye.

In the absence of any further orbital receptors capable of feedback there remains a much discussed possibility of corollary discharge whereby the brain records the pattern strength of motor activity used in executing eye movements (Helmholtz, 1867). Some comments on corollary discharge have been made in the introduction and it would be inappropriate to discuss the subject any further here.

4.5 THE POTENTIAL FUNCTION OF PROPRIOCEPTION IN HUMAN EOM.

Attempts to demonstrate a stretch reflex (Keller and Robinson, 1971) from the human EOM have resulted in the contention that if such a reflex exists at all, its contribution to muscle tone must be a feeble one. In view of the role in the reflex loop which has been credited to the muscle spindle, the absence of a stretch reflex is consistent with the findings of phylogenetically redundant spindles in human EOM. Although functional muscle spindles are essential for the monosynaptic reflex loop, the absence of a stretch reflex is insufficient evidence to exclude them as a potential source of proprioception. Polysynaptic pathways to higher regions of the central nervous system could, for instance, provide conscious sense of the position of our eyes irrespective of the lack of a motor response. In this respect it is of interest that patients suffering from active herpes zoster ophthalmicus experience an impaired spatial localisation in the active phase of the condition (Campos et al,1986) which could be attributed to disturbance of proprioception. But although indicative of an proprioceptive influence on specification of visual direction, these results do not prove that such a sensation necessarily originates from muscle tissue.

Steinbach and Smith (1981) suggested that the musculotendinous region contained proprioceptors important for specifying eye position. This suggestion was based on the finding that patients who had had the same muscles operated on in repeated procedures did not appear to have inflow information about eye position, presumably as a consequence of damage to tendon receptors. Examination of the site of surgery led to the finding of palisade endings (Richmond et al, 1984). If, as shown here, tendons are without receptors in young subjects the deficit noted by these authors must be attributed to some other cause. The peculiar morphological features of the receptors in human EOM seem to argue against a normal proprioceptive function, but there are nevertheless strong reasons to believe that some sort of extra retinal source, providing information about eye position, does exist. In the literature, the most favoured evidence of such a source seems to be the visual system's ability to differentiate between shifts in the retinal image caused by object movements versus eye rotations. During a voluntary eye movement , fixed objects in the visual field appear stationary, and do not move in the opposite direction as might be expected from the consequent displacement of the retinal image. In order to achieve this the visual information must somehow be compared with information regarding eye movements. It is not difficult to see how the spindle could play a vital role in such a process but the current data provides little evidence to substantiate such a role. Simple physiological experiments also indicate that muscle proprioception cannot possible provide stability of the visual world. This can best be demonstrated by passive movement of the globe (Bridgeman and Stark, 1991). Rotation of the globe by external forces will initiate no efferent activity. Changes in muscle length should, however, still initiate an afferent signal by deformation of the primary sensory ending of the intrafusal muscle fibres. The apparent motion of the visual world which most observers perceive during such an experiment indicates that proprioception does not decrease the perception of motion. As long as the involuntary rotation of the globe and corresponding

displacement of the retinal image is fairly consistent with the apparent movement of the visual world, any existing suppression initiated by proprioception must necessarily be minute.

An increasing number of clinical trials have shown that trained observers fail to report any substantial sense of their eye position and the inflow input in general, conscious or subconscious, seems to contribute little to motor control of the eye (Carpenter, 1988).

CONCLUSION

Bimodality of extra ocular muscle nerve fibre diameters has previously been reported by others. A third peak is now added by including unmyelinated fibres which constitute a substantial number of the total.
The functional implication of this observation suggests that Felderstruktur fibres which are known to be served by such small diameter efferents have a finer motor control than previously assumed.

There is an apparent decrease in the size of the motor unit with age due to a reduction in the number of muscle fibres. Such changes, which have not been reported elsewhere, might contribute to some of the deficits in motility seen in old age.

The peculiar features of muscle spindles in the infant human EOM are similar to those reported earlier in mature individuals and indicate an incapacity to fulfil a

proprioceptive function and promote the view that they are phylogenetically redundant.

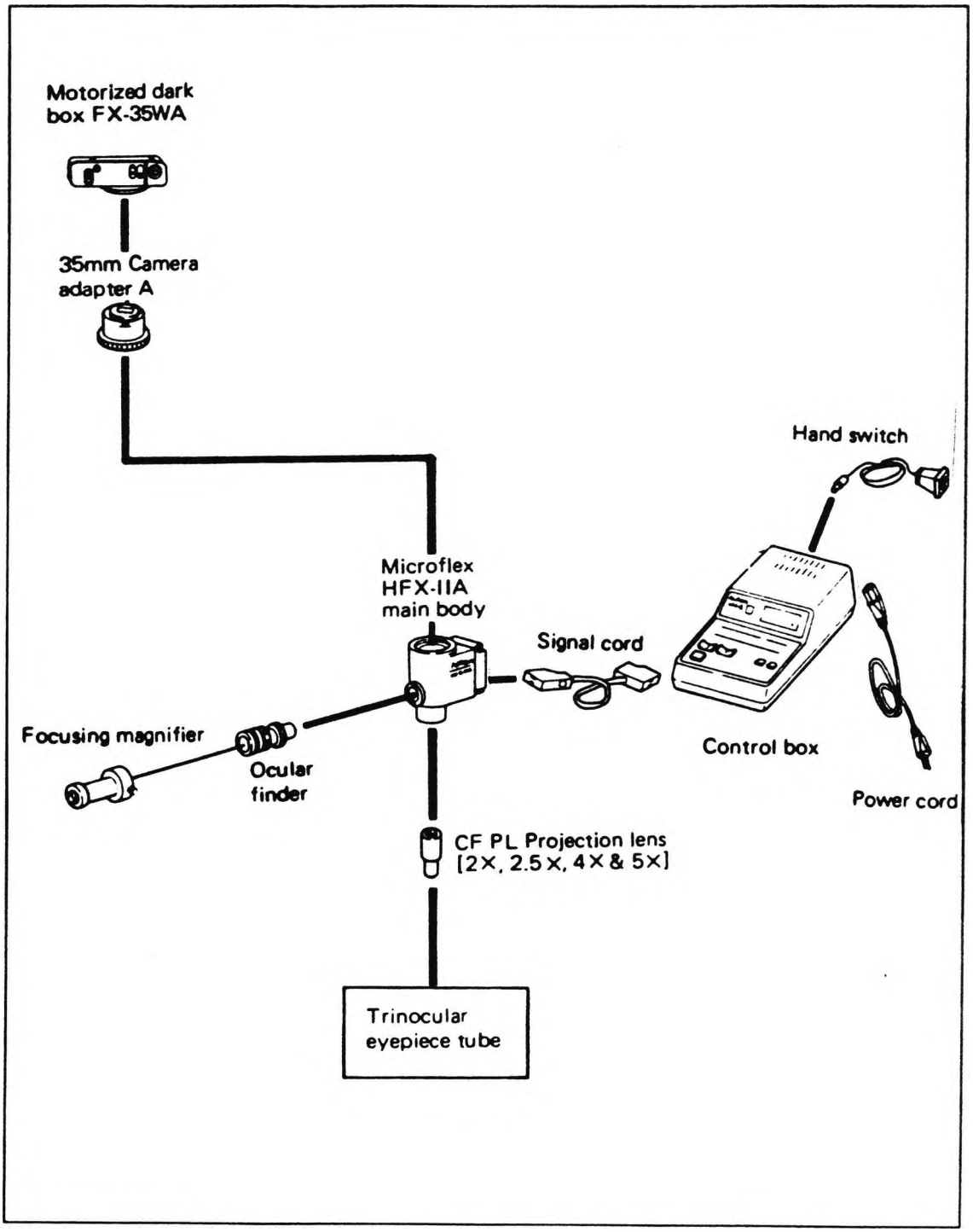
Tendon receptors without the characteristics of GTO but with morphological features expected of receptors were found. But the delay in their appearance until maturity rules out the possibility that they might play a vital role in the development of binocular vision. Further, it is difficult to conceive of a functional role applicable exclusively to mature EOM.

The highest number of tendon receptors was found in muscles displaying most signs of myofilament loss and Ringbinden fibres. Although arguable, the concept of afferent proliferation induced by structural age-related changes seems highly unlikely. To suggest that the acquired myotendinous endings are in anyway compensatory for these changes is therefore intangible.

The ability to distinguish between retinal displacement of an image by object movement and eye movement (displacement-cancellation hypothesis) would seem to require information about the position and movement of the eye. If, as it appears, muscle receptors cannot provide this information one must look for other sources. The case for efference copy as a provider of eye position information is strengthened.

APPENDIX

APPENDIX A
Technical information on the microflex HFX-IIA system

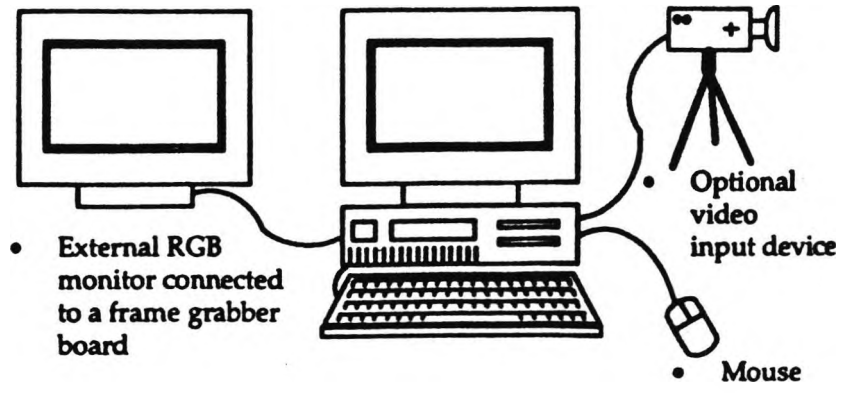


APPENDIX B
Global LAB image technical information

System Requirements (With a Dual Monitor Frame Grabber)

GLOBAL LAB *Image* supports image capture and image display on an external monitor when used with DT55, DT53, DT2867, and DT2867-LC boards.

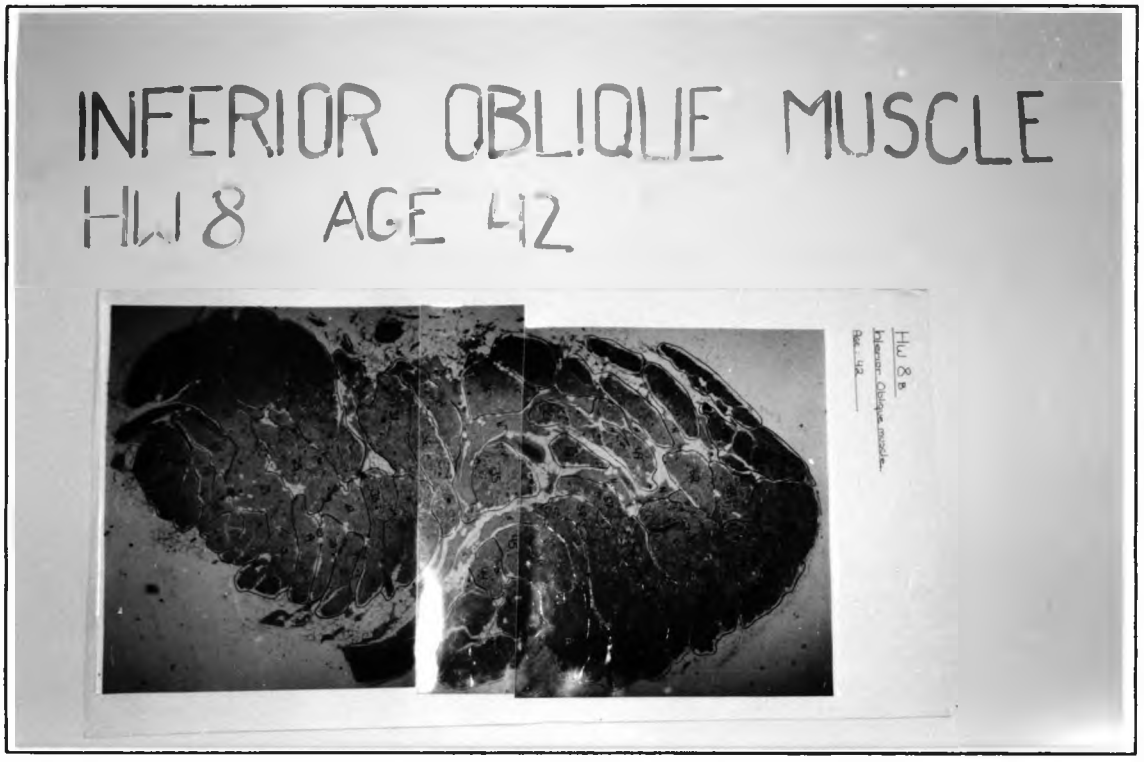
◇ *Note: The DT55 and DT53 were previously called DT2855 and DT2953, respectively.*

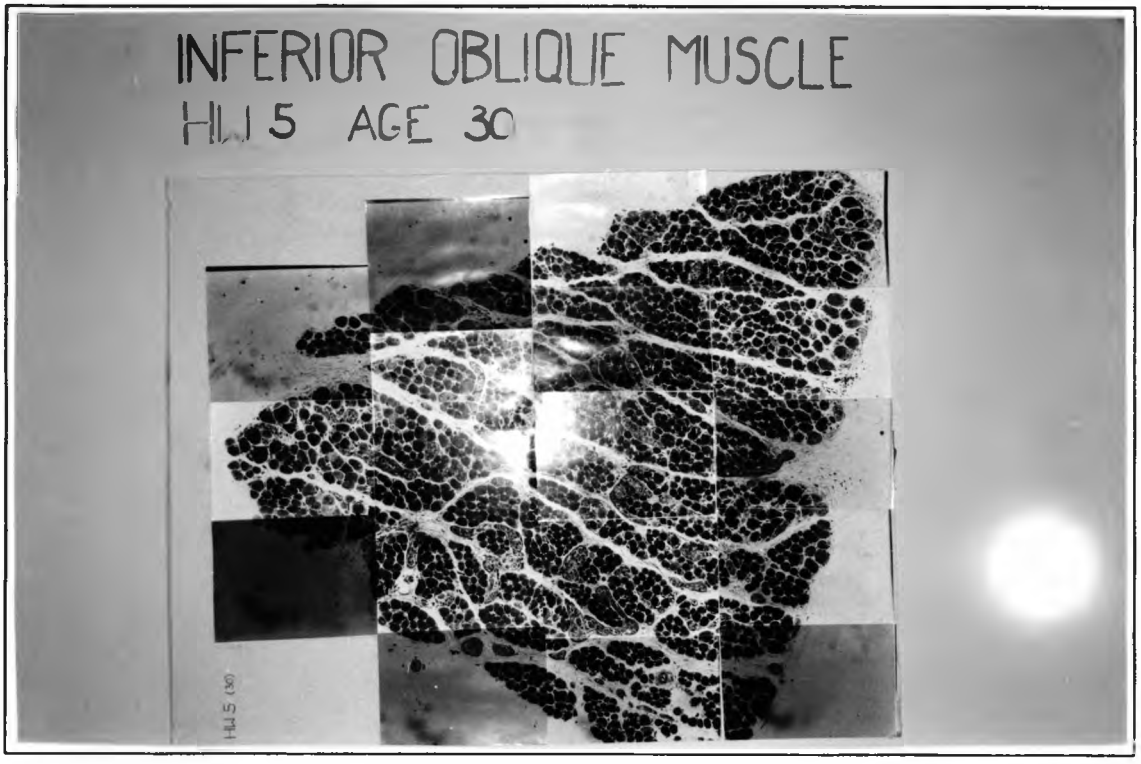


- A 16-bit ISA 80386- or 80486-based PC
- A Windows-compatible display adapter
- At least one 5.25-inch or 3.5-inch high density floppy disk drive and one 20 Mbyte hard disk drive
- 4 Mbytes of free extended memory (8 or more Mbytes strongly recommended)
- Compatible frame grabber
- Microsoft Windows 3.1
- MS-DOS or PC-DOS operating system Version 3.3 or later
- With an 80386-based PC, an 80387 math coprocessor is recommended but not required

APPENDIX C

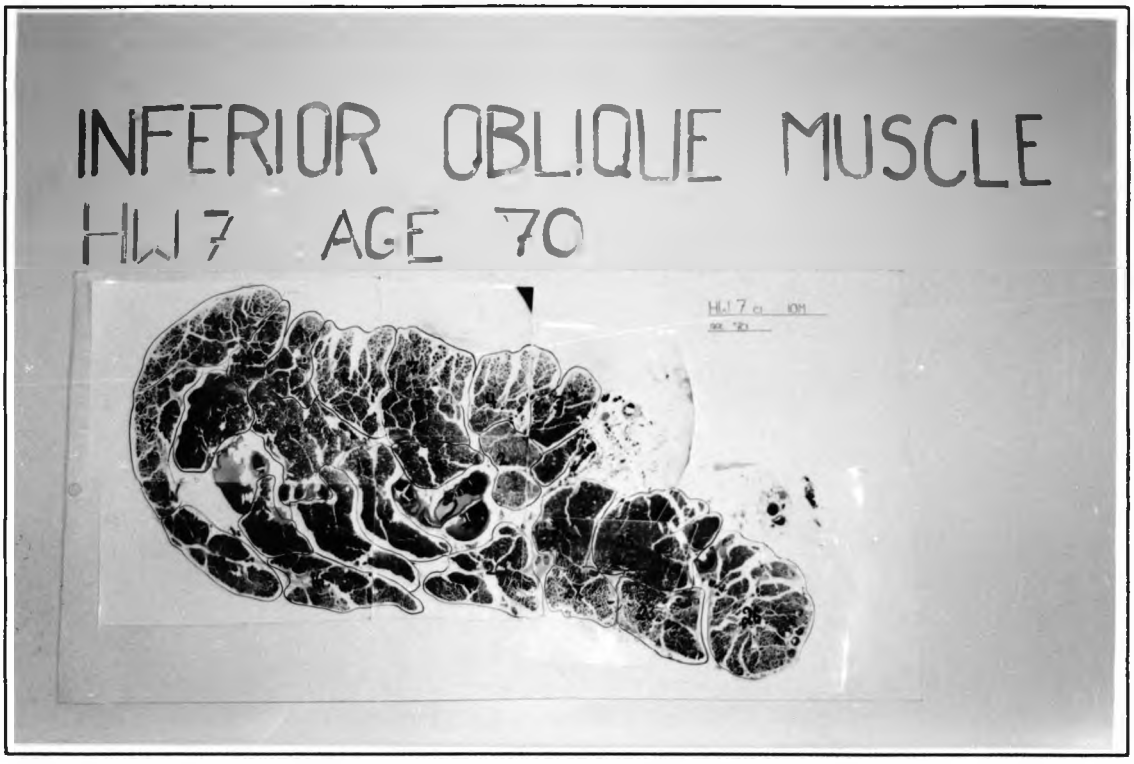
Photomontages of human EOM. Each montage was made from light micrographs of low magnification. Stained with toluidine blue.
Reference number and age of subject as indicated.

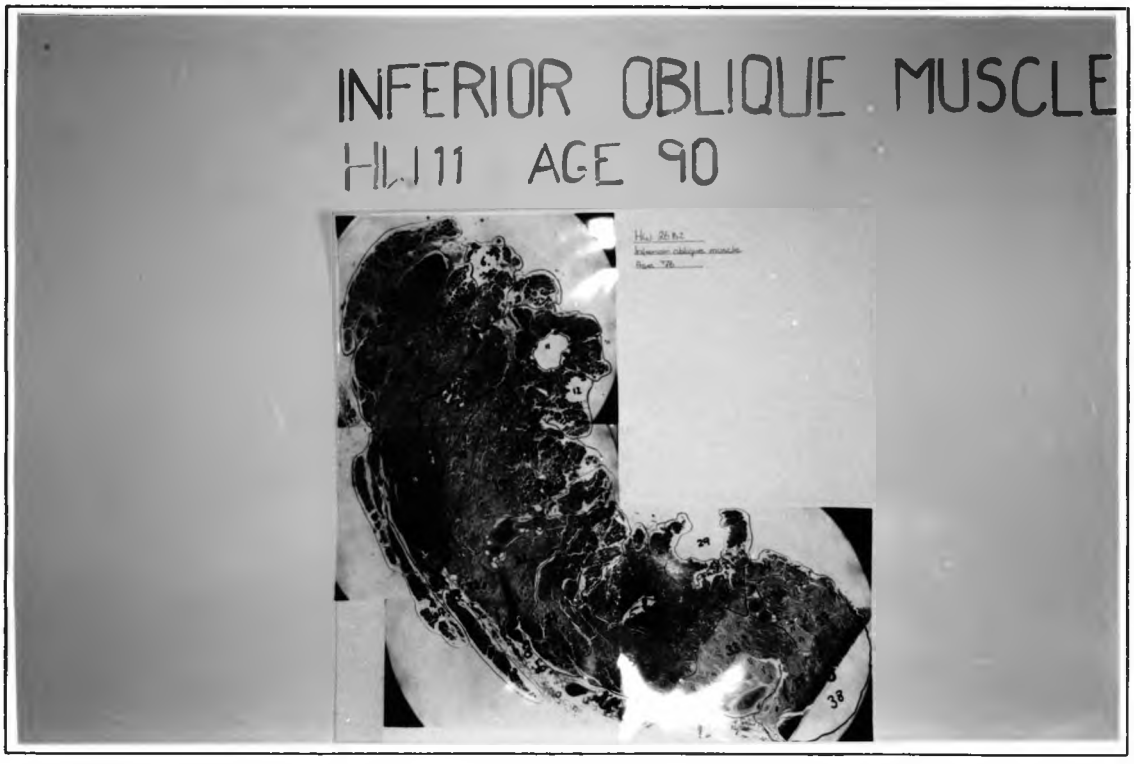




APPENDIX C

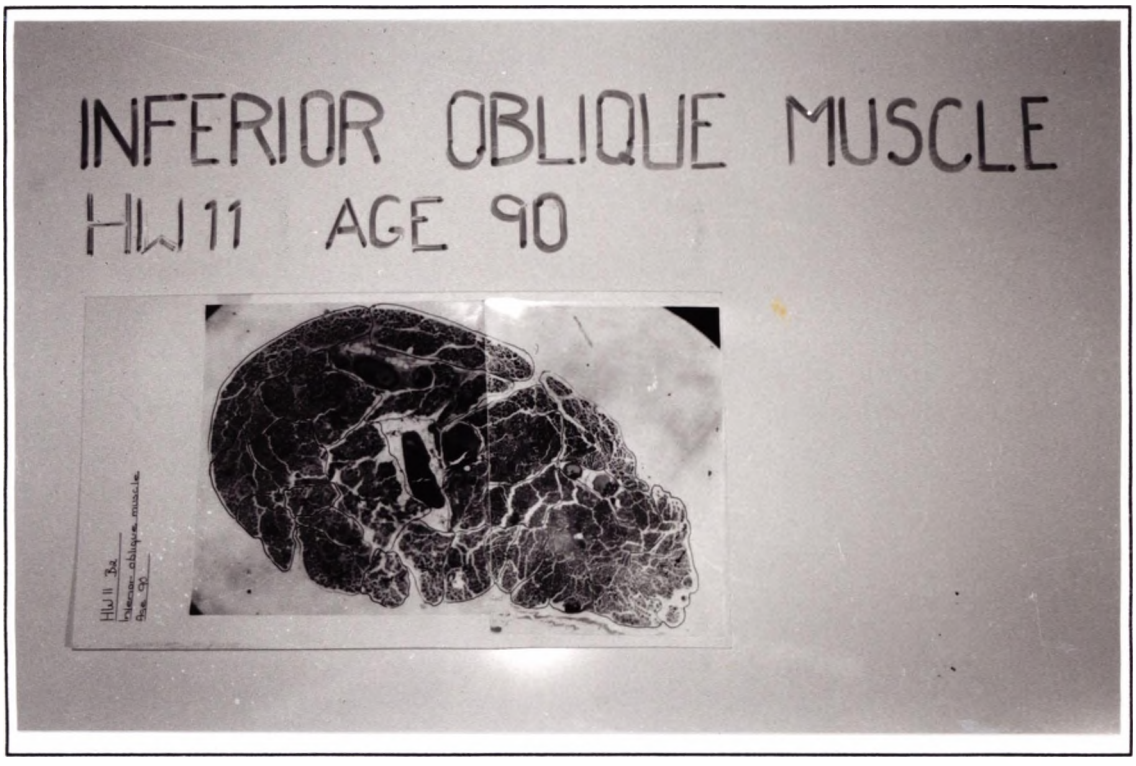
Photomontages of human EOM. Each montage was made from light micrographs of low magnification. Stained with toluidine blue.
Reference number and age of subject as indicated.





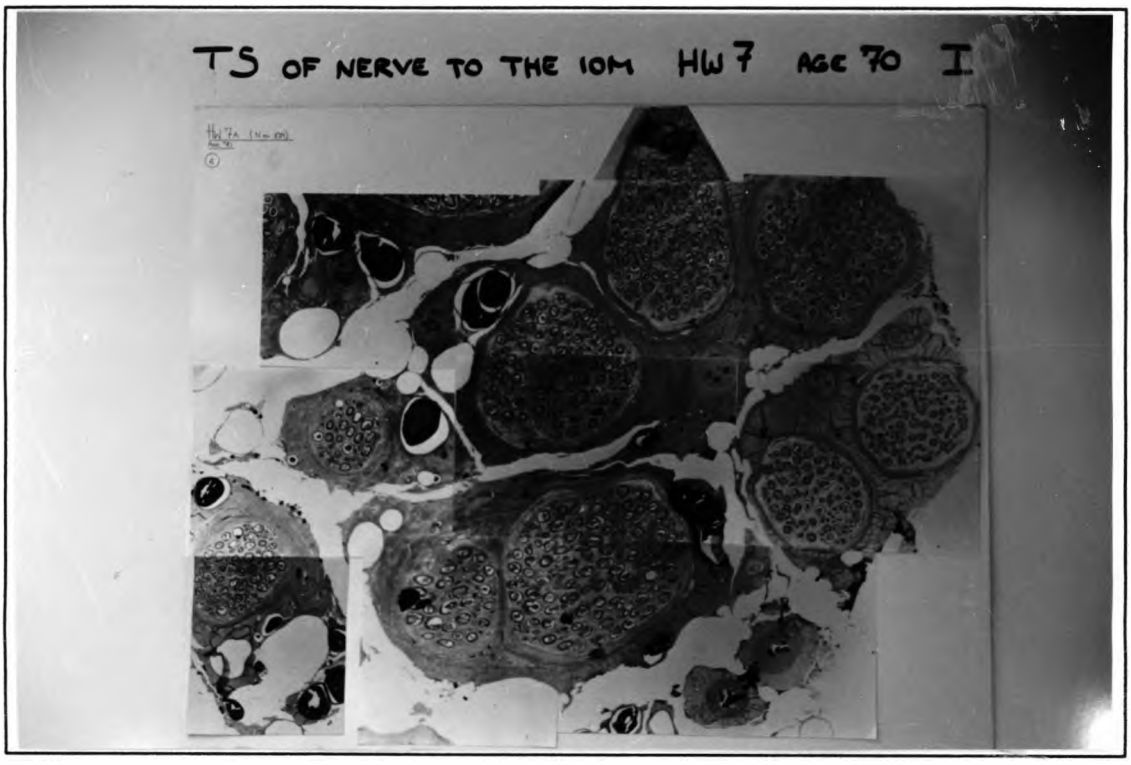
APPENDIX C

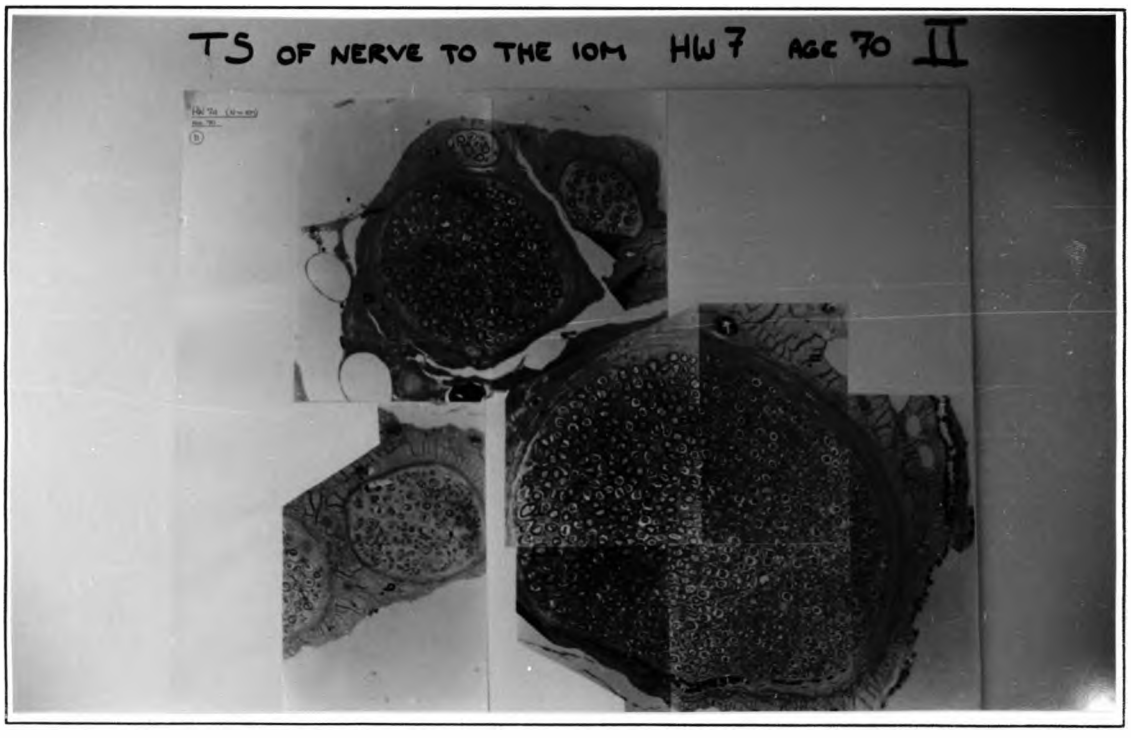
Photomontages of human EOM. Each montage was made from light micrographs of low magnification. Stained with toluidine blue.
Reference number and age of subject as indicated.



APPENDIX D

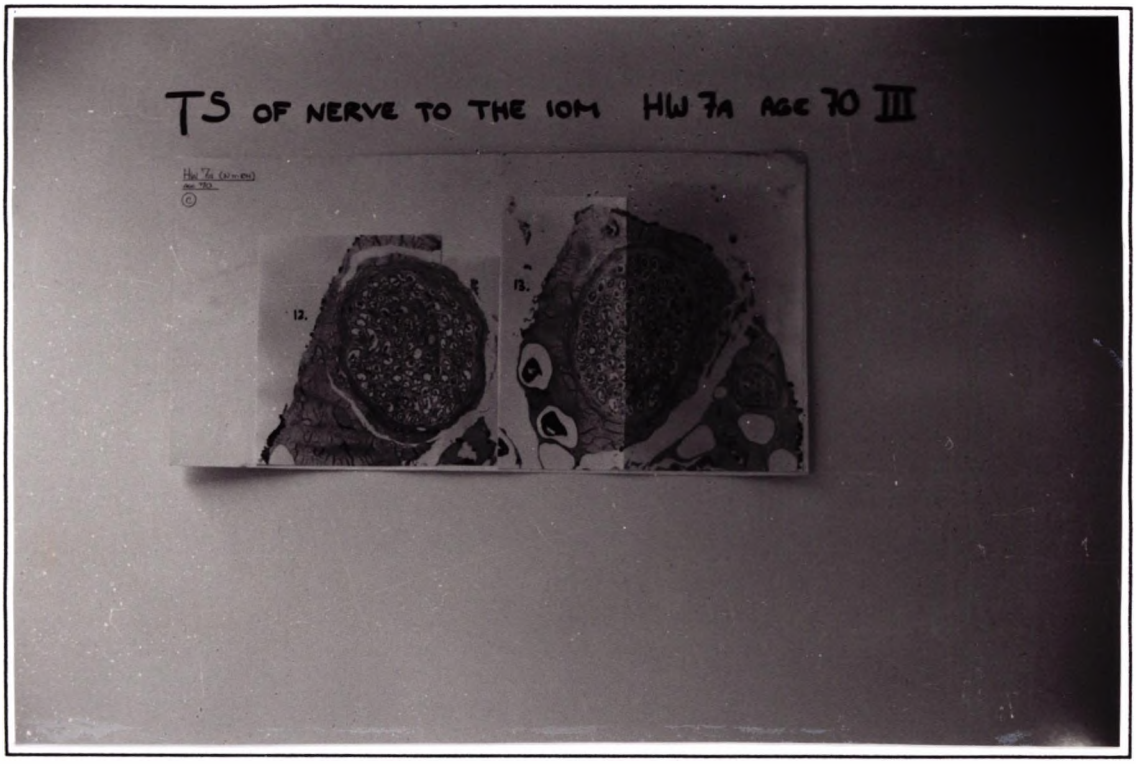
Photomontages of nerves to the inferior oblique muscle in transverse section.
Original magnification of the photomontage is 640.
Reference number and age of subject as indicated.

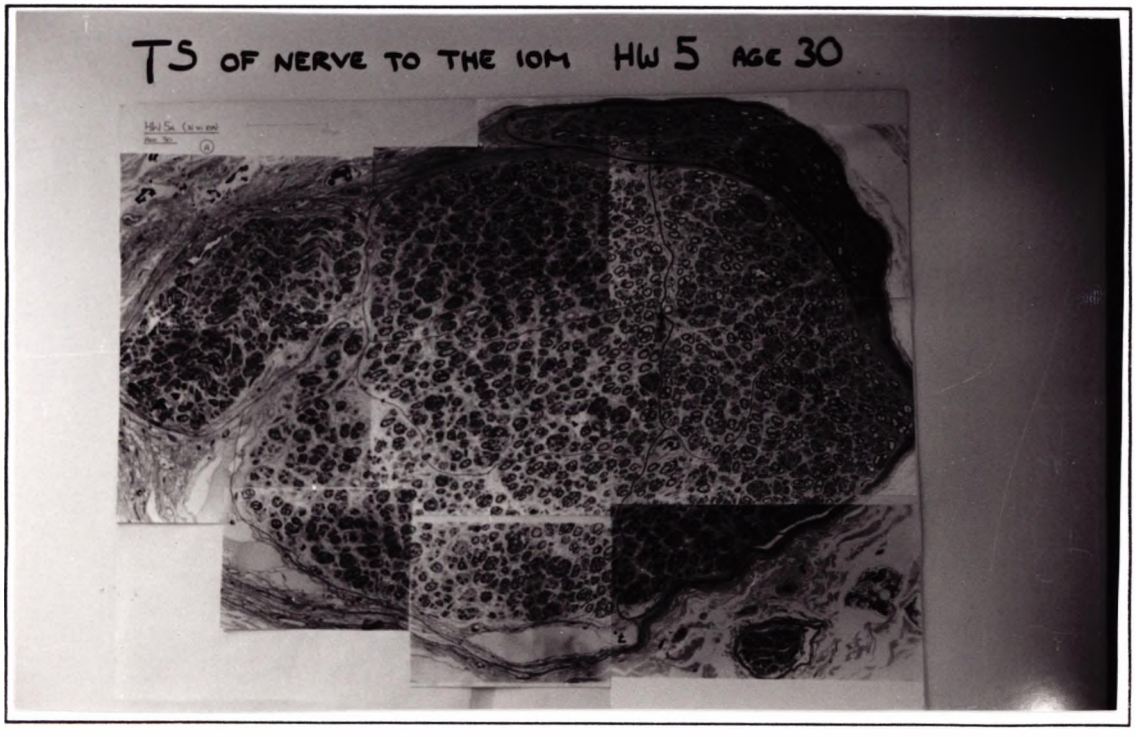




APPENDIX D

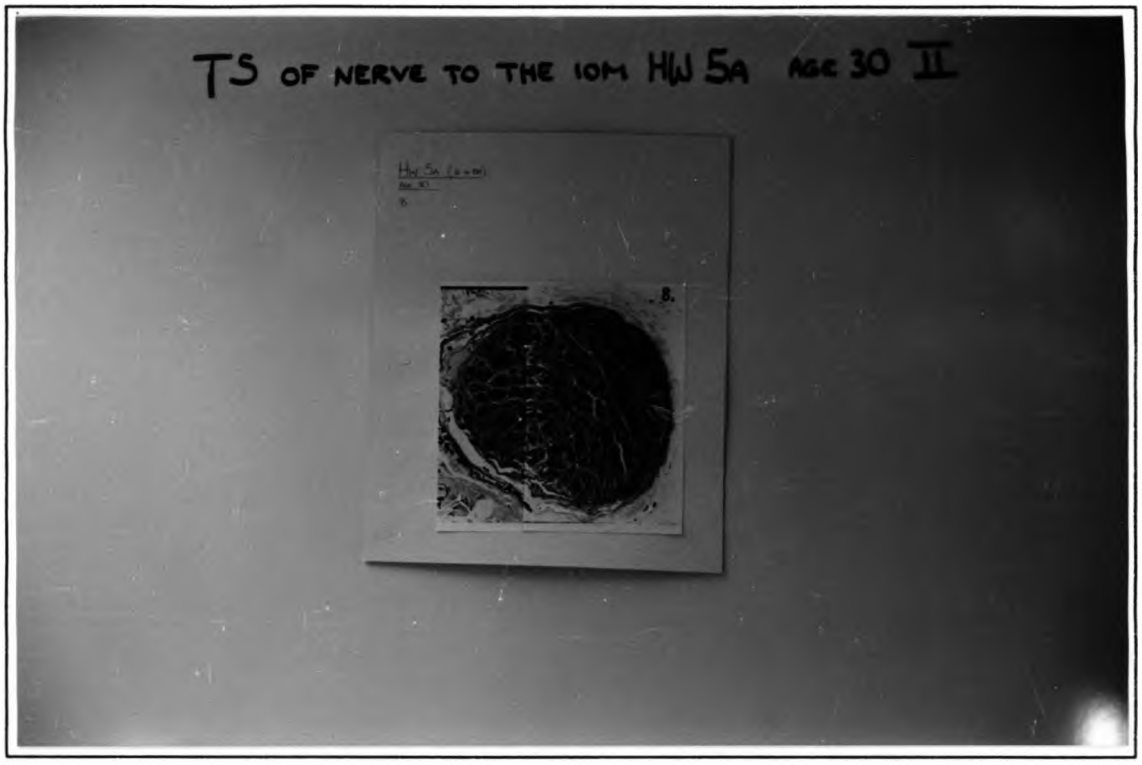
Photomontages of nerves to the inferior oblique muscle in transverse section.
Original magnification of the photomontage is 640.
Reference number and age of subject as indicated.

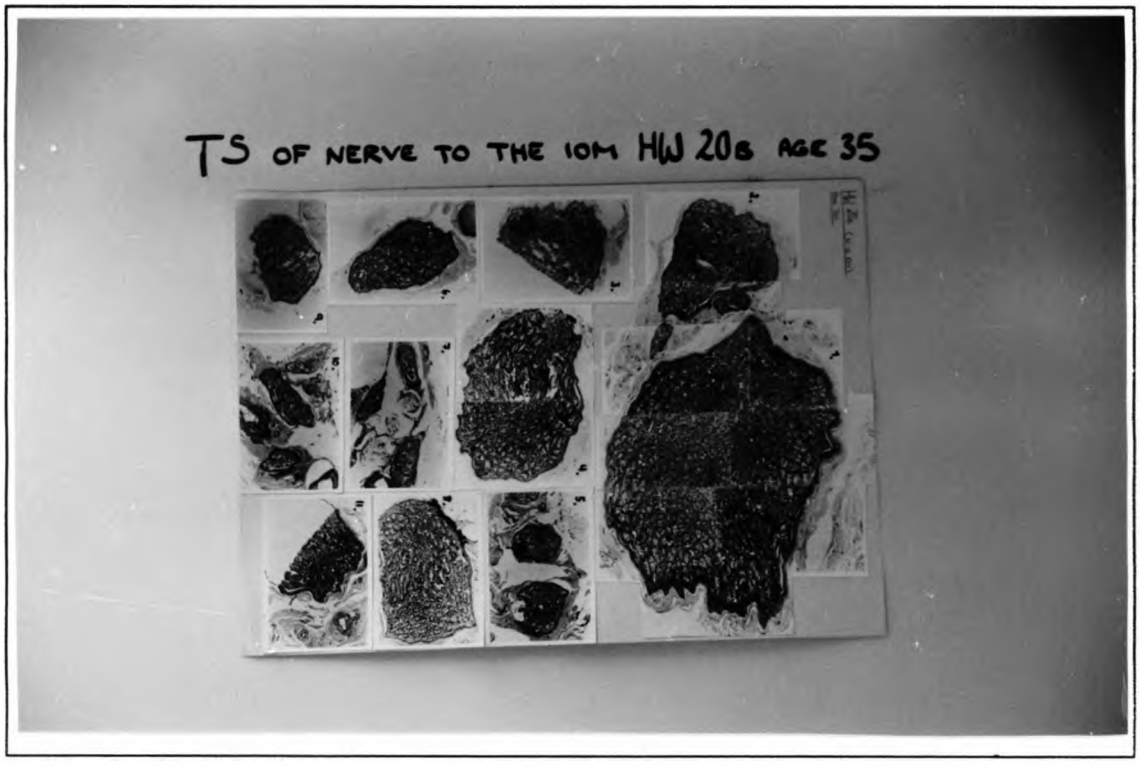




APPENDIX D

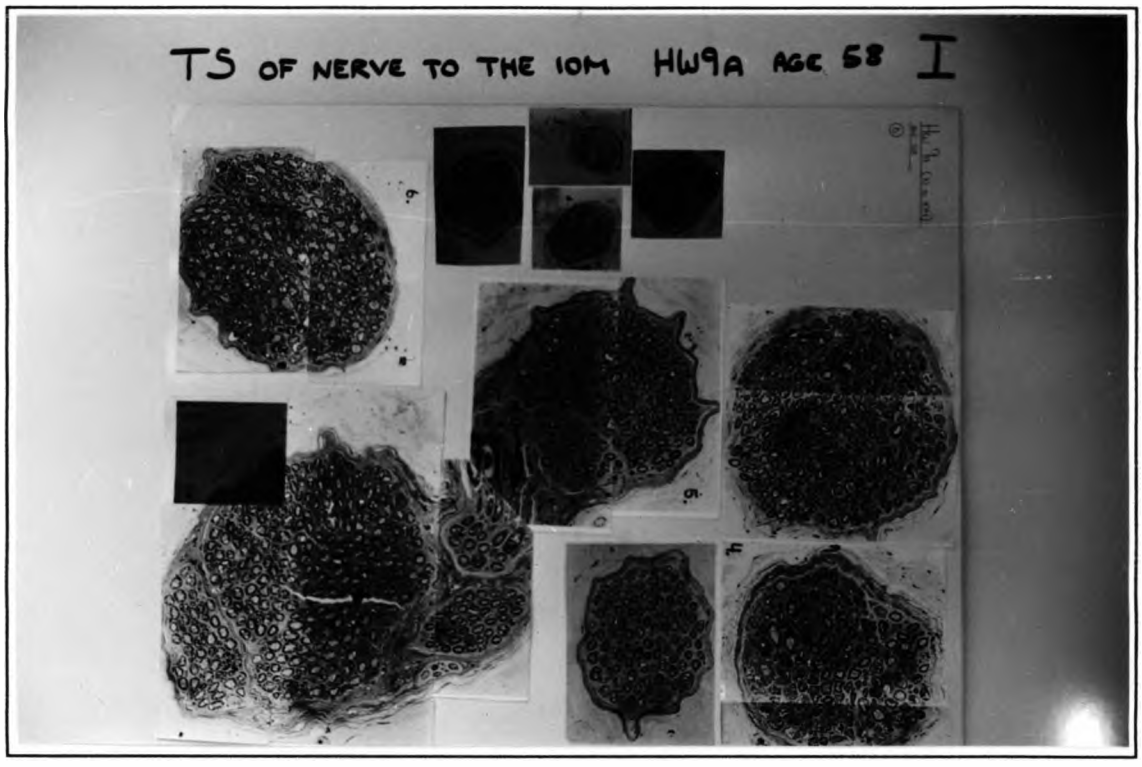
Photomontages of nerves to the inferior oblique muscle in transverse section.
Original magnification of the photomontage is 640.
Reference number and age of subject as indicated.

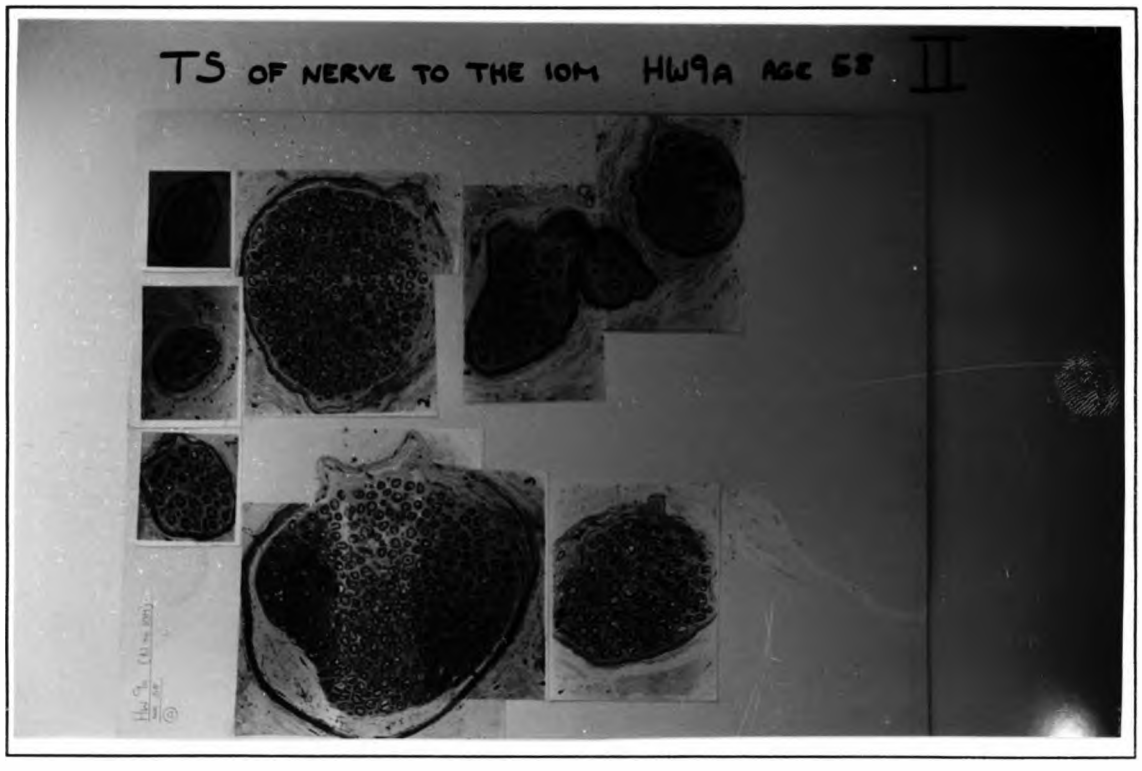




APPENDIX D

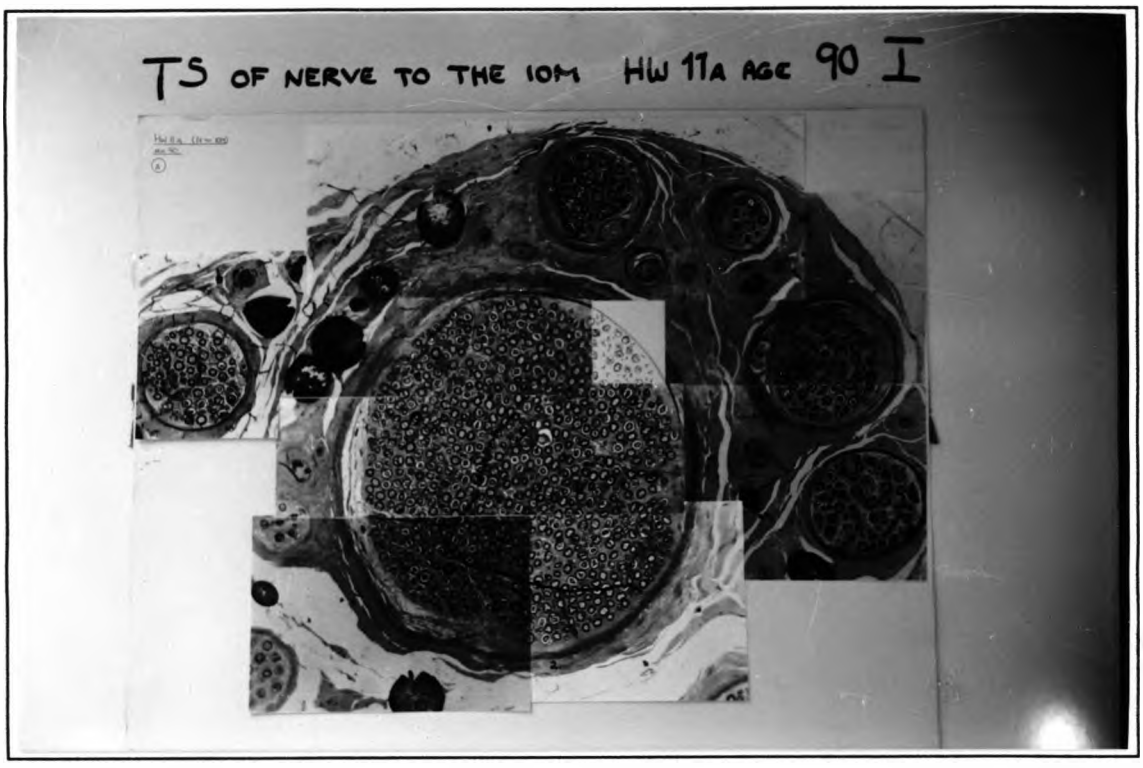
Photomontages of nerves to the inferior oblique muscle in transverse section.
Original magnification of the photomontage is 640.
Reference number and age of subject as indicated.

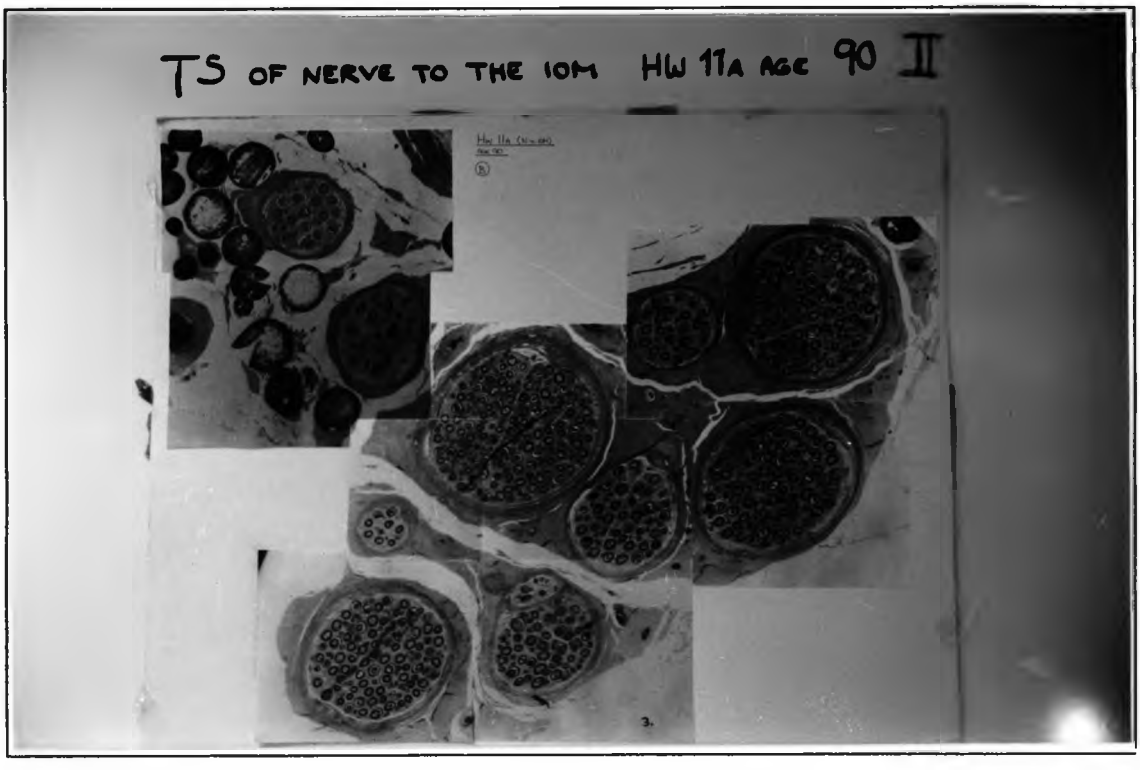




APPENDIX D

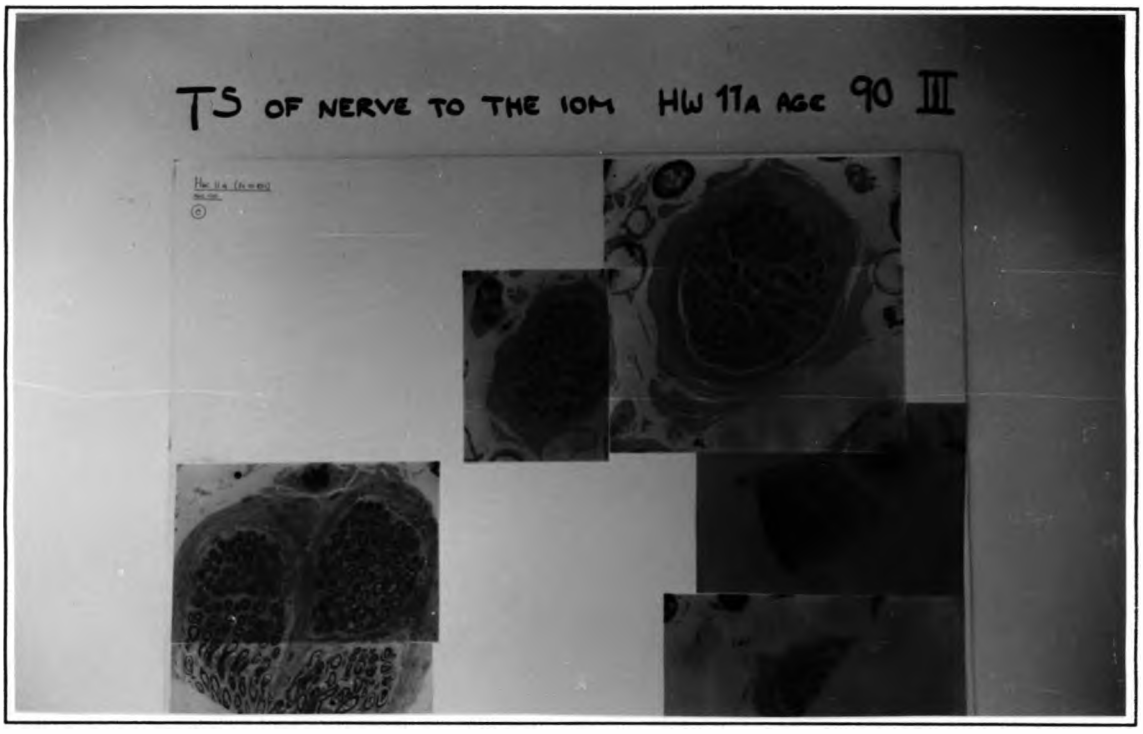
Photomontages of nerves to the inferior oblique muscle in transverse section.
Original magnification of the photomontage is 640.
Reference number and age of subject as indicated.

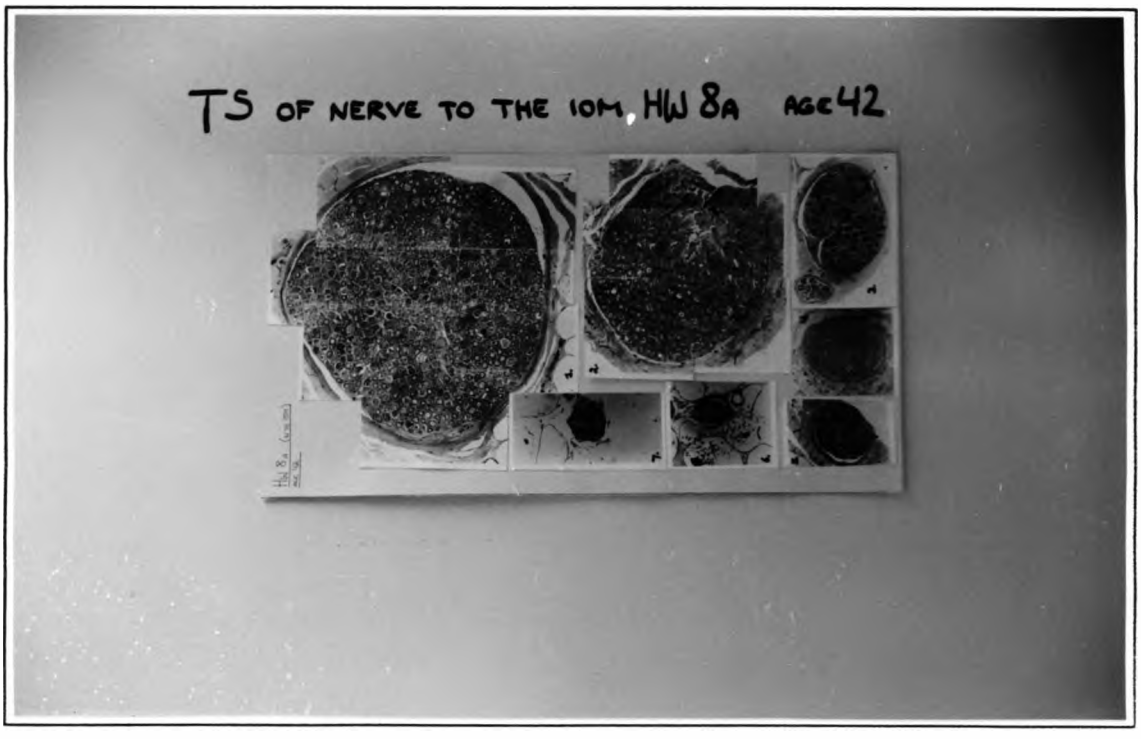




APPENDIX D

Photomontages of nerves to the inferior oblique muscle in transverse section.
Original magnification of the photomontage is 640.
Reference number and age of subject as indicated.





APPENDIX E Table showing dimensions of muscle spindles and their sources.

No.	Subject reference and age	Type of muscle	Spindle length	Spindle diameter
1	HC5 23 months	IOM	532 μm	75μm
2	HC5 23 months	IOM	450 μm	75μm
3	HC5 23 months	IOM	285 μm	50 μm
4	HC5 23 months	IOM	247 μm	25*75 μm (elongated)
5	HC2 5 months	IOM	112 μm	25 μm
6	HC2 5 months	IOM	173 μm	24 μm
7	HC2 5 months	IOM	135 μm (false spindle)	25*75 μm (elongated)
8	HC2 5 months	IOM	173 μm (false spindle)	25 μm)
9	HC2 5 months	IOM	105 μm (false spindle)	50*75 μm (elongated)
10	HC6 30 months	IOM	135 μm (false spindle)	25*75 μm (elongated)
11	HC6 30 months	IOM	38 μm (false spindle)	45 μm
12	HC6 30 months	IOM	75 μm (false spindle)	12 μm
13	HC6 30 months	IOM	187 μm	35 μm
14	HC6 30 months	IOM	338 μm	75*50 μm (elongated)
15	HC2 5 months	MRM	510 μm	49 μm

APPENDIX E ctd.

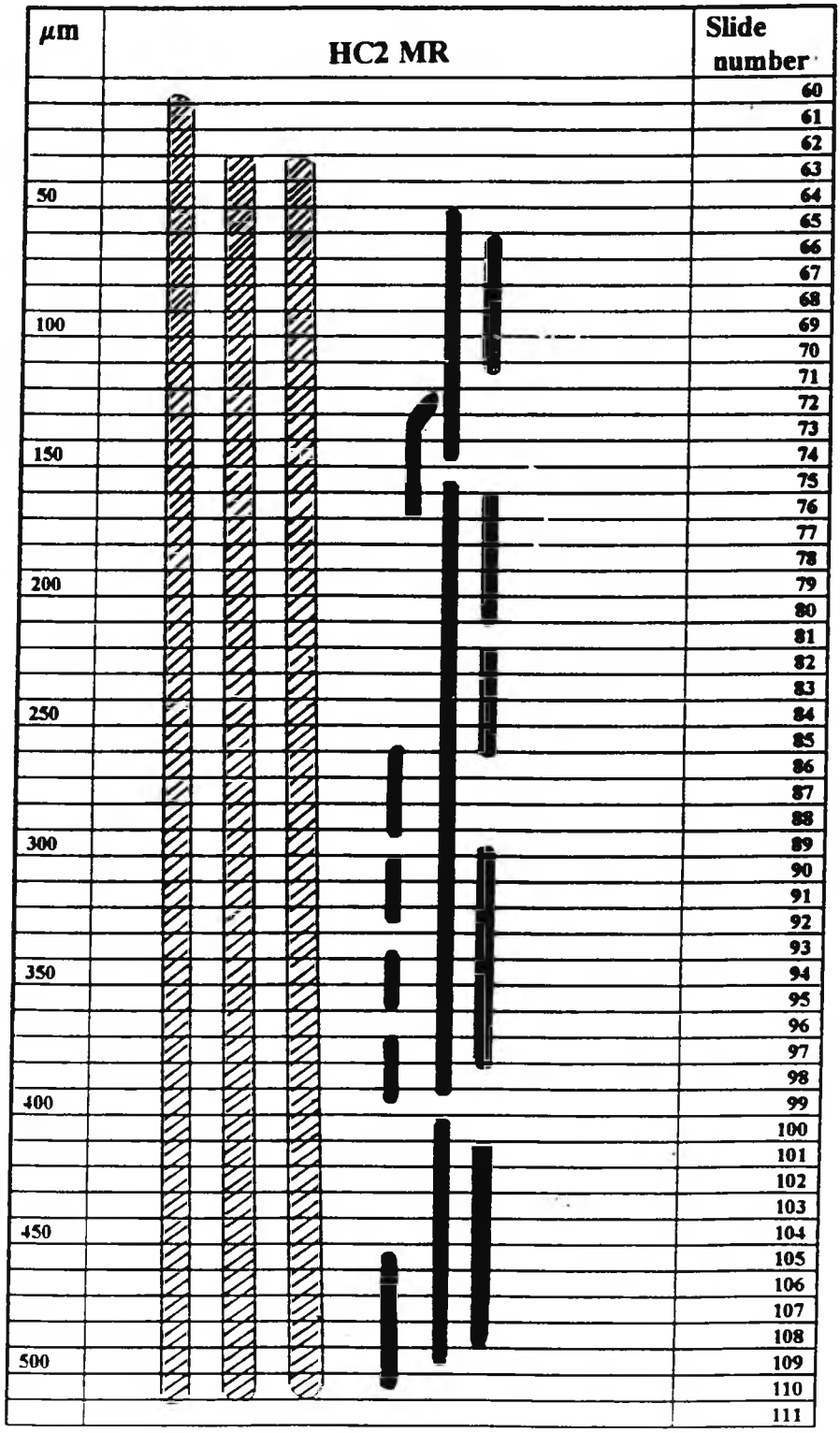
No.	Subject reference and age	Type of muscle	Spindle length	Spindle diameter
16	HC2 5 months	MRM	375 μm (false spindle)	20 μm
17	HC2 5 months	MRM	428 μm	103 μm
18	HC2 5 months	MRM	150 μm	27 μm
19	HC2 5 months	MRM	157 μm	22 μm
20	HC2 5 months	MRM	383 μm	60 μm
21	HC2 5 months	MRM	293 μm	73 μm
22	HC2 5 months	MRM	428 μm	70 μm
23	HC2 5 months	MRM	615 μm	53 μm
24	HC2 5 months	MRM	458 μm	97 μm
26	HC2 5 months	MRM	248 μm	63 μm
27	HC2 5 months	MRM	233 μm	67 μm
28	HC2 5 months	MRM	683 μm	72 μm
29	HC2 5 months	MRM	308 μm	25 μm
30	HC2 5 months	MRM	307 μm	53 μm
31	HC2 5 months	MRM	510 μm	105 μm

APPENDIX E ctd.

No.	Subject reference and age	Type of muscle	Spindle length	Spindle diameter
32	HC4 6 days	MRM	45 μm	34 μm
33	HC4 6 days	MRM	143 μm	30 μm
34	HC4 6 days	MRM	90 μm	40 μm
35	HC4 6 days	MRM	105 μm	36 μm
36	HC4 6 days	MRM	38 μm	62 μm
37	HC4 6 days	MRM	165 μm	54 μm
38	HC4 6 days	MRM	143 μm	50 μm
39	HC4 6 days	MRM	128 μm	64 μm
40	HC4 6 days	MRM	225 μm	74 μm

APPENDIX F

Scheme of a representative muscle spindle illustrating the various intrafusal fibres. Cross-hatched thick lines represent the fibres with extrafusal features without equatorial modification. Solid thin lines represent fragmented or foreshortened fibres. Reference number and length of fibres as indicated.



APPENDIX G

Computer print outs from the analysis of multiple innervated fibre with Fibrillenstruktur features

1. (Slide 101 and 102) Single efferent axon in the process of dividing.

2. (Slide 105,108 and 113) The axon divides and gives off one major branch which encircles the muscle fibre.

3. (Slide 105) The axon terminates on a typical motor end plate (MEP).

4. (Slide 124) The remaining branch continues further along the length of the fibre towards a second motor end plate.

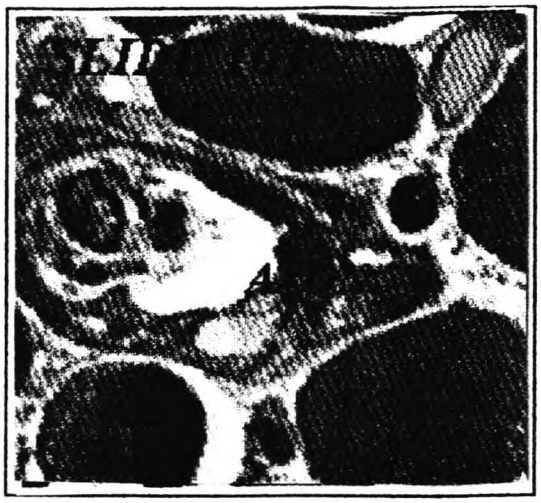
5. (Slide 141, 142 and 144) Single axon parallel to muscle fibre.

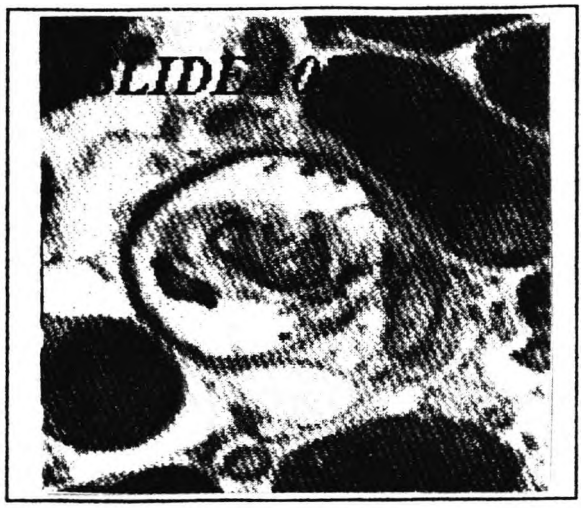
6. (Slide 146 and 148) Motor endplate displaying axoplasm and sole plate nuclei.

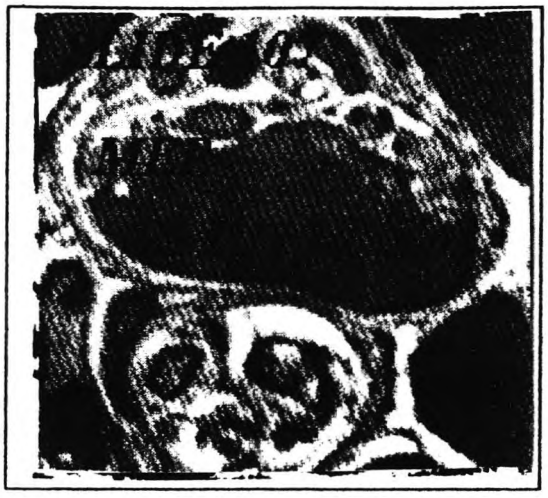
7. (Slide 150) Terminal boutons of the second motor end plate.

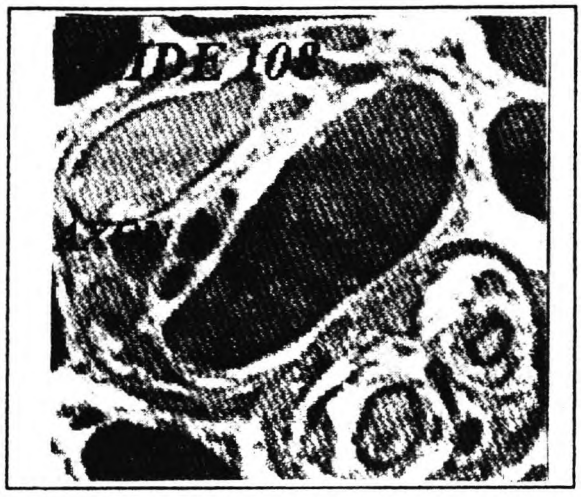
APPENDIX G
Computer print outs from the analysis of multiple innervated fibres with Fibrillenstruktur features

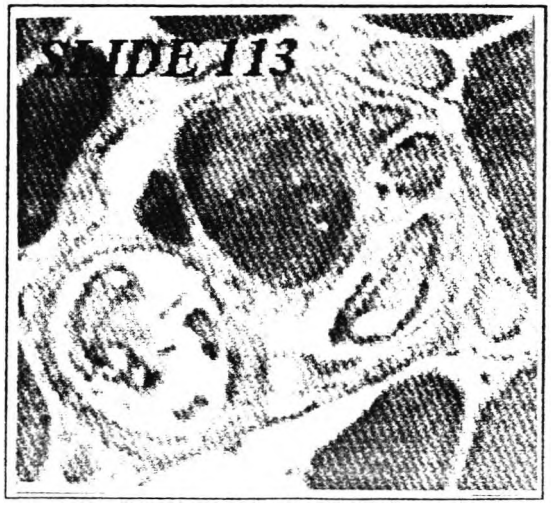
HW 11 IOM 90

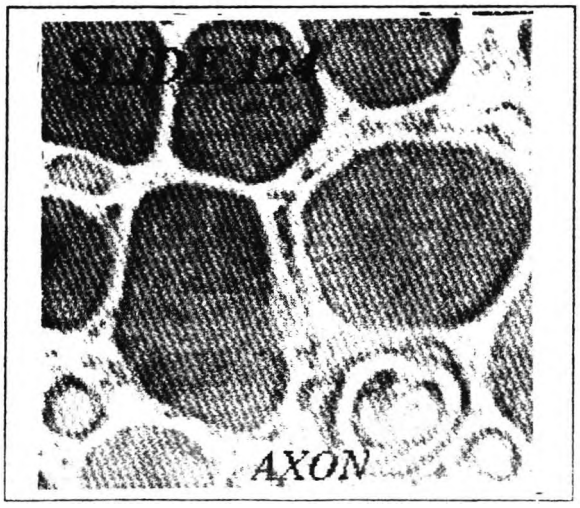








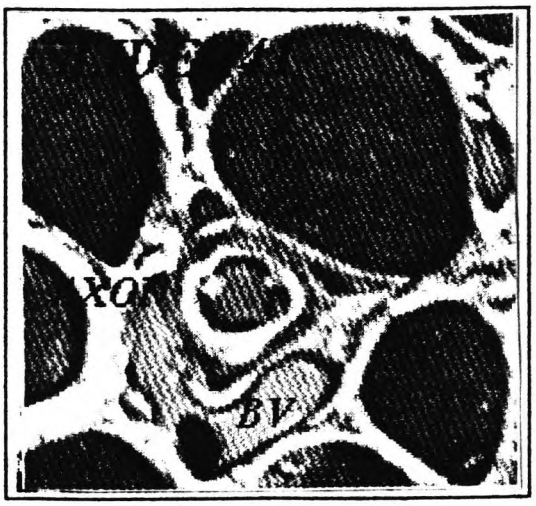


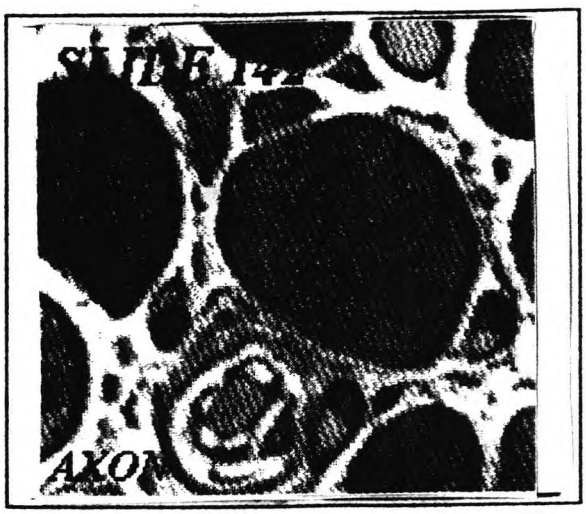


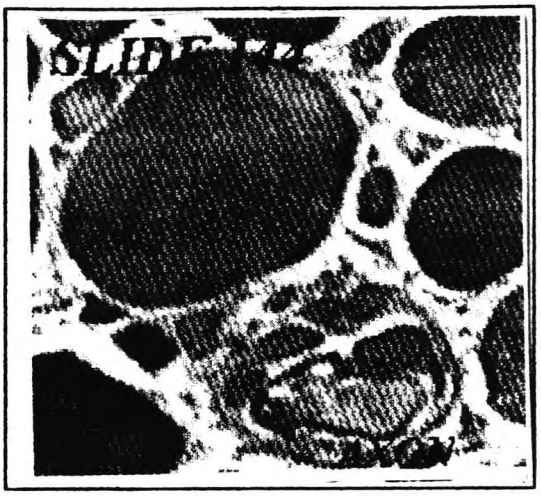
APPENDIX G

Computer print outs from the analysis of multiple innervated fibres with Fibrillenstruktur features

HW 11 IOM 90







REFERENCES

Alvarado, J.A and Van Horn, C. (1974). Muscle cell types of the cat inferior oblique. In Basic Mechanisms of Ocular Motility and Their Clinical Implications (eds. Lennerstrand,G. and Bach-y-Rita, P), pp 15-43. Pergamon Press, Oxford..

Alvarado- Mallart, R.-M., & Pincon-Raymond, M. (1979) The pallisade ending of cat extraocular muscles: A light and electron microscope study. Tissue & Cell **11,** 567-584

Bach-y-Rita, P (1971) Neurophysiology of eye movements. In The control of Eye Movements (eds Bach-y-Rita, P., Collins, C.C. and Hyde, J.E) pp 7-45. Academic press, New York

Bach-y-Rita, P. & Ito, F. (1966). In vivo studies on fast and slow muscle fibres in cat extraocular muscles. Journal of General Physiology **49,** 1177-1198

Bach-y-Rita, P. & Lennerstrand G. (1974). Absence of polyneural innervation in cat extraocular muscles. Journal of Physiology **244,** 613-624

Baichenko, P.I., Matyushkin, D.P. and Suvorov V.U. (1968) Participation of fast and tonic oculomotor systems in stretch reflexes and labyrinthine reflexes. Neuroscience Translation **3,** 350-358.

Barker,D., (1974). The morphology of muscle receptors. In Muscle Receptors. (ed. Hunt) pp 1-188. Springer

Batini, C & Buisseret, P. (1974). Sensory peripheral pathway for extrinsic eye muscles Archives Italiennes de Biologie **112,** 18-32

Bors, E. (1926). Über das zahlenverhältnis zwischen nerven und muskelfasern. Anatomischer Anzeiger **60,** 415-416

Brandt, D.B. & Leeson, C.R. (1966). Structural differences of fast and slow fibres in human extraocular muscles. American Journal of Ophthalmology **62,** 478-487

Breinin, G.M., (1957). Electromyographic Evidence of Ocular Muscle Proprioception in Man. Archives of Ophthalmology **57,** 176-180

Breinin, G.M. (1971) The structure and function of extraocular muscle- An apraisal of the duality concept. American Journal of Ophthalmology **72,** 1-9

Bridgeman, B. & Stark, L. (1991) Ocular proprioception and efference copy in registering visual direction. Vision Research **11,** 1903-1991

Brodal (1990) The sensory system. In Structure and Function of the Central Nervous System.. pp 109-176. TANO A.S, Oslo

Brzezinski, D.K. (1961) Untersuchungen zur Histochemie der Muskelspindeln. II. Mitteilung: zur Topochemie und Funktion des Spindelraumes und der Spindelkapsel. Acta histochemica **12,** 277-288

Büttner, U. & Büttner-Ennever, J.A. (1988) Present concepts of oculomotor organisation. In Neuroanatomy of the Oculomotor system (ed. Büttner-Ennever, U), pp. 1- 23. Elsevier, New York

Büttner-Ennever, J.A. & Akert, K. (1981) Medial rectus subgroups of the oculomotor nucleus and their abducens internuclear input in the monkey. Journal of Comparative Neurology **197,** 17-27

Buzzard, E. F., (1908) A note on the occurrence of muscle-spindles in ocular Muscles. Proceedings of the Royal Society of Medicine **1,** 83-88

Campos , E.C., Chiesi, C. & Bolzani, R. (1986) Abnormal spatial localisation in patients with herpez zoster ophthalmicus. Archives of Ophthalmology **104,** 1176-1177

Carpenter (1988) Statics and Dynamics of the Eye. In Movements of the Eye (ed Carpenter) pp 139-178 Pion Limited, London

Cheng, K. (1963) Cholinesterase activity in human extraocular muscles. Japanese Journal of Ophthalmology **7,** 174-184

Chamberlain, W. (1971) Restriction in upwards gaze with advancing age. American Journal of Ophthalmology **71,** 341-346

Chiarandini, D.J. & Davidowitz, J. (1979) Structure and function of extraocular muscle fibers. In Current Topics in Eye Research. Volume 1, (eds Zadunaisky, J.A. and Davson, H.) pp 91-143. Academic Press

Collins, C.C. (1971) Orbital mechanics. In The Control of Eye Movements (eds Bach-y-Rita, P., Collins, C.C. and Hyde, J.E.) pp 285-325 Academic Press, New York

Collins, C.C, O'Meara, D., Scott, A.B. (1975) Muscle tension during unrestrained human eye movements. Journal of Physiology **245,** 351-369

Cooper, S. & Daniel, P.M. (1949) Muscle spindles in human extrinsic eye muscles. Brain **72,** 1-24

Cooper, S., Daniel, P.M. & Whitteridge, D. (1955) Muscle spindles and other sensory endings in the extrinsic eye muscles; the physiology and anatomy of these receptors and of their connexions with the brain stem. Brain **78,** 564-583

Davidowitz, J., Chiarandini, D.J., Philips, G. & Breinin, G.M. (1981) Morphological variation along multiply innervated fibres in rat extraocular muscles. In Functional Basis of Ocular Motility Disorders (eds Lennerstrand, G., Zee, D.S. and Keller, E.L.), pp 17-26 Pergamon Press, Oxford

Dietert, S.E. (1965) The demonstration of different types of muscle fibers in human extraocular muscle by electron microscopy and cholinesterase staining. Investigative Ophthalmology **4,** 51-63

Dogiel , A.S. (1906) Die endungen der sensibilen Nerven in den Augenmuskeln und deren Sehnen beim Menschen und den Saugetieren. Archiv für microskopische Anatomie und Entwicklungsmechanik **68,** 501-526

Eggers, H.M. (1982) Functional anatomy of the extraocular muscles. In Ocular Anatomy, Embryology and Teratology (ed. Jakobiec, F.A.), pp 783-824. Harper & Row, Oxford

Feinstein, B., Lindegård, B., Nyman, E. & Wohlfart, G. (1954) Morphologic studies of motor units in normal human muscles. Acta Anatomica **23,** 127-142

Fernand, V.S.V. & Hess, A. (1969) The occurrence, structure and innervation of slow and twitch muscle fibres in the tensor tympani and stapedius of the cat. Journal of Physiology **200,** 547-554

Fiorentini, A. & Maffei, L. (1977) Instability of the eye in the dark and proprioception. Nature **128,** 182-99

Gamble, H.J., Fenton, J. & Allsopp, G. (1978) Electron microscope observations on human fetal striated muscle. Journal of Anatomy **126,** 567-589

Goldschmitd, M. (1969) Beitrag zur anatomie des Musculus rectus externus und des Musculus rectus internus bei Hund und Mensch. Ophthalmologica **157,** 381-390

Golgi, C. (1880) Sui nervi dei tendini dell'uomo e di altri vertebrati e di un nuovo organo nervoso terminale musculo-tendineo. Memorie della Reale Academia delle Scienze di Torino Serie II, Vol XXXII. (Abstract in English)

Granit, R. (1971) The probable role of muscle spindles and tendon organs in eye movement control. In The control of Eye Movements. (eds Bach-y-Rita, P., Collins, C.C. and Hyde, J.E.) , pp 3-5 Academic Press, New York

Hain, A. & Diamond R. (1983) Contribution of eye movement to the representation in space. In Spatially Orientated Behaviour (ed Hain, A. & Jeannerod, M.) pp 119-133, Springer

Helmholtz, H. von, (1867) Handbuch der Physiologischen Optik 1st edition (Voss, Hamburg) 3rd edition translated by J.P.C. Southall for the Optical Society of America (1924)

Hess, A (1961) The structure of slow and fast extrafusal muscle fibres in the extraocular muscles and teir nerve endings in guinea pigs. Journal of Cellular and Comparative Physiology. 58, 63-80

Hess, A. & Pilar, G. (1963) Slow fibres in the extraocular muscle of the cat. Journal of Physiology **169,** 780-788

Holland, G. (1956) Untersuchongen uber den einfluss des alters auf die physiologischen grenzen der augenbeweglichkeit. Klinische Monatsblatter fur Augenheilkunde **129,** 655-662

Hoogenraad, T.U., Jennekens, F.G.I., & Tan, K.E.W.P. (1979) Histochemical fibre types in human extraocular muscles, an investigation of inferior oblique muscle. Acta Neuropathologica **45,** 73-78

Hunt, C.C. (1974) Muscle Receptors. In Handbook of Sensory Physiology Volume III/2 (ed Hunt, C.C.) Springer, Berlin

Kato, T. (1938) Über histologische Untersuchungen der Augenmuskeln von Menschen und Saugetieren Okajamas Folia Anatomica Japonicae **16,** 131-145

Keller, E.L. & Robinson, D.A. (1971) Absence of a stretch reflex in extraocular muscle of the monkey. Journal of Neurophysiology **34,** 908-919

Krüger, P. (1949) Die innervation der tetanischen und tonischen Fasern der quergestreiften Skeletmuskulatur der Wirbeltiere. Anatomischer Anzeiger **97,** 169-175

Kuffler, S.W. & Williams, E.M.V. (1953) Small-nerve potentials. The distribution of small motor nerves to frog skeletal muscle, and membrane characteristics of the fibres they innervate. Journal of Physiology **121,** 289-317

Larsson, L. & Ansved, T. (1995) Effects of ageing on the motor unit. Progress in Neurobiology **45,** 397-458

Lawrensen, J.G. & Ruskell, G.L.(1991) The structure of corpuscular nerve endings in the limbal conjunctiva of the human eye. Journal of Anatomy **177,** 75-84

Länergren, J. (1975) Structure and function of twitch and slow fibres in amphibian skeletal muscle. In Basic Mechanisms of Ocular Motility and their Clinical implications (eds Lennerstrand G. and Bach-Y-Rita, P.) pp 63-84. Pergamon, Oxford

Lee, J. (1994) Personal communication at the VIII Scandinavian Orthoptic meeting. Stavanger, Norway.

Lennerstrand, D. (1982) Postnatal development of eye muscle function : An experimental study in cats with normal and impaired binocular vision. In Functional Basis of Ocular Motility Disorders. (eds Lennerstrand G., Zee, D.S., and Keller, E.L.) pp 39-47. Pergamon, Oxford.

Lennerstrand, G. (1975) Motor units in eye muscles. In Basic Mechanisms of Ocular Motility and their Clinical implications (eds Lennerstrand, G. and Bach-Y-Rita, P.) pp119-144. Pergamon, Oxford

Locket, N. A. (1968) The dual nature of human extraocular muscles. British Orthoptic Journal **25,** 2-11

Lucas, J.R., Aigner, M., Blumer, R., Heinzl, H. and Mayr, R. (1994) Number and distribution of the neuromuscular spindles in human extraocular muscle. Investigative Ophthalmology & Visual Science **35,** 4317-4327.

Manni, E. & Bortolami, R. (1981) Proprioception in eye muscles. In Functional Basis of Ocular Motility Disorders (eds. Lennerstrand, G., Zee, D.S. and Keller,E.L.) pp 55- 67 Pergamon, Oxford

Martinez, A.J., Hay, S. & McNeer, W. (1976) Extraocular muscles light microscopy and ultrastructural features. Acta Neuropathologica **34,** 237-253

Matin, L. (1986) Visual localisation and eye movements. In Handbook of Perception and Human Performance (eds Boff, K.R., Kaufmann, L. & Thomas, J.P.) Vol 1 Sensory Processes and Perception, pp 1-46. Wiley, New York

McComas, A.J. (1991) Invited review: Motor unit estimation: Methods, results and present status. Muscle and Nerve **14,** 585-597

McNeer, K.W. & Spencer, R.F. (1981) The histopathology of human strabismic extraocular muscle. In Functional Basis of Ocular Motility Disorders (eds Lennerstrand, G., Zee, D.S., and Keller, E.L.) pp 27-38. Pergamon, Oxford

Merrilles, N.C.R., Sunderland, S. & Hayhow, W. (1950) Neuromuscular spindles in the extraocular muscles in man. Anatomical Record **108,** 23-30

Merton, P.A. (1961) The accuracy of directing the eyes and hand in the dark. Journal of Physiology **156,** 555-577

Miller, N.R. (1985) Central trigeminal pathways of the extraocular muscle spindles. In Walsh & Hoyt's Clinical Neuro-Ophthalmology (ed Miller, N.R.) pp 1030-1032 Williams & Wilkins, Baltimore

Miller, J.E. (1975) Ageing changes in extraocular muscle. In Basic Mechanisms of Ocular Motility and their Clinical implications (eds Lennerstrand, G. & Bach-Y-Rita, P.) pp 47-61 Pergamon, Oxford

Mitsui, Y. (1986) Strabismus and the Sensorimotor Reflex. Excerpta Medica, Tokyo

Mühlendyck, H. and Ali, S.S. (1978) Histological and ultrastructural studies on the Ringbands in human extraocular muscles. Graefes Archiv Ophthalmologie **208,** 177-191

Mukuno, K. (1967) The fine structure of the human extraocular muscles. Part 2 The classification of muscle fibres. Japanese Journal of Ophthalmology **12,** 9-20

Mukuno, K. (1968) Fine structure of the human extraocular muscles. Part 3. Neuromuscular junction in the normal human extraocular muscles. Acta Societatis ophthalmologicae japonicae **72,** 104-121 (In Japanese)

Mukuno, K. (1983) Neuro-ophthalmology of the extraocular muscles. Fibre type, proprioception, denervation. 87 th Annual Meeting of Japanese ophthalmological Society.

Noorden von, G.K. (1990) Summary of the Gross Anatomy of the Extraocular muscles. In Binocular Vision and Ocular Motility (ed von Noorden, G.K.) pp 45 Mosby St. Louis

Nunomura, S., Hizawa, K., Ii, K. & Sano, T. (1984) A histochemical study on fibre types in human extraocular muscles. Biomedical Research **5,** 295-302

Optican, L.M. & Zee, D.S. (1984) A hypothetical explanation of congenital nystagmus. Biological Cybernetics **50,** 119-134

Peachey, L. (1971) The structure of the extraocular muscle fibres in mammals. In The Control of Eye Movements. (eds Bach-y-Rita,P., Collins, C.C. and Hyde, J.E.) pp 47-66 Academic press, New York.

Pilar, G. (1967) Further study of the electrical and mechanical responses of slow fibres in cat extraocular muscles. Journal of General Physiology **50,** 2289-2300

Porter, J.D. (1986) Brainstem termination of extraocular muscle primary afferent neurons in the monkey. Journal of Comparative Neurology **247,** 133-143.

Porter, J.D. & Baker, R. (1992) Prenatal morphogenesis of primate extraocular muscle: neuromuscular junction formation and fiber type differentiation. Investigative Ophthalmology & Visual Science **33,** 657-670.

Porter, J.D., Baker, R.S., Ragusa, R.J. and Brueckner, J.K. (1995) Extraocular muscles: basic and clinical aspects of structure and function. Survey of Ophthalmology. **39,** 451-484

Porter, J.D. & Donaldson, M.L. (1991) The anatomical substrate for cat extraocular muscle proprioception. Neuroscience **43,** 473-481

Porter, J.D. and Spencer, R.F. (1982) Localisation and morphology of cat extraocular muscle afferent neurons identified by retrograde transport of horseradish peroxidase. Journal of Comparative Neurology **204,** 56-64

Richmond, F. J. R., Johnson, W. S. W., Baker, R. S. & Steinbach, M. J. (1984) Palisade endings in human extraocular muscles. Investigative Ophthalmology and Visual Science **25,** 471-476

Ringel, S.R., Wilson, B., Barden, M.T. & Kaiser, K.K. (1978) Histochemistry of human extraocular muscle. Archives of Ophthalmology **96,** 1067-1072

Ruskell , G.L (1990) Golgi tendon organs in the proximal tendon of sheep extraocular muscles. Anatomical Record **227,** 25-31

Ruskell, G.L (1989) The fine structure of human extraocular muscle spindles and their potentional proprioceptive capacity. Journal of Anatomy. **167,** 199-214

Ruskell, G.L. (1978) The fine structure of innervated myotendinous cylinders in extraocular muscles of rhesus monkeys. Journal of Neurocytology **7,** 693-708

Ruskell, G.L. (1979) The incidence and variety of Golgi tendon organs in extraocular muscles of the rhesus monkey. Journal of Neurocytology **8,** 639-653

Ruskell, G.L. (1983) Fibre analysis of the nerve to the inferior oblique muscle in monkeys. Journal of Anatomy **137,** 445-455

Ruskell, G.L. (1983) Spiral nerve endings in human extra ocular muscles terminate in motor end plates. Journal of Anatomy **139,** 33-43

Ruskell,G.L (1984) Sheathing of muscle fibres at neuromuscular junctions and at extra-junctional loci in human extraocular muscles. Journal of Anatomy **138,** 33-44

Sadeh, M. and Stern, L.Z. (1984) Observations on the innervation of human extraocular muscles. Journal of the Neurological Sciences **66,** 295-305

Salpeter, M.M. (1987) The vertebrate neuromuscular junction . In Neurology and Neurobiology (eds Chan-Palay & Palay) Alan R. Liss Inc., New York

Schoultz, T.W., & Swett, J.E. (1972) Ultrastructural organisation of the sensory fibres innervating the Golgi tendon organ. Anatomical Record **179,** 147-162

Scott, A.B. (1971) Active force tests in lateral rectus paralysis. Archives of Ophthalmology **85,** 397-404

Scott, A.B. & Collins, C.C. (1973) Division of labour in human extraocular muscles. Archives of Ophthalmology **90,** 319-322

Sevel, D. (1981) A reappraisal of the origin of the human extraocular muscles. Ophthalmology **88,** 1330-1338

Sherrington, C.S. (1918) Observations on the sensual role of the proprioceptive nerve supply of the extrinsic ocular muscles. Brain **41,** 332-343

Sherrington, C.S. (1925) Remarks on some aspects of reflex inhibition Proceedings of the Royal . Soc. London 97,519-545

Skavenski, A.A. (1972) Inflow as a source of extraretinal eye position information. Vision Research **12,** 221-229

Sodi, A., Corsi, M., Faussone Pellegrini, M.S. & Salvi, G. (1988) Fine structure of the receptors at the myotendinous junction of human extraocular muscles. Histology and Histopathology **3,** 103-113

Spencer, F. & Porter,D. (1988) Structural organisation of the extraocular muscles. In Neuroanatomy of the Oculomotor System (ed Buttner-Ennever, J.A.) pp 33-79 Elsevier Science Publishers, Amsterdam

Sperry, R. W., (1950) Neural basis of the spontaneous optokinetic response produced by visual inversion. Journal of Comparative Psychology and Physiology **43,** 482-489

Steinbach, M. J. & Smith, D.R. (1981) Spatial localisation after strabismus surgery: evidence for inflow. Science **213,** 1407-1409

Teräväinen, H. (1968) Electron microscopic and histochemical observations on different types of nerve endings in the extraocular muscles of the rat. Zeitschrift für Zellforschung und Mikroskopische Anatomie **90,** 372-388

Tergast, P. (1873) Ueber das Verhältniss von Nerve und Muskel. Archiv für Mikroskopische Anatomie **9,** 36-46

Thulin, I. (1914) Histologie des muscles oculaires chez l'homme et les singes. Societa Biologia **76,** 490-493

Tomlinson, B.E. and Irving, D. (1977) The number of limb motor neurons in the human lumbosacral cord throughout life. Journal of the Neurological Sciences **34,** 213-219

Torre, M. (1953) Nombre et dimensions des unités motrices dans les muscles extrinséques de loeil et, en général, dans les muscles squélettiques reliés á des organes de sens. Schweizer Archiv fuer Neurologie und Psychiatrie **72,** 362-376

Trotter, Y., Fregnac, Y. & Buisseret, P. (1987) The period of susceptibility of visual cortical binocularity to unilateral proprioceptive deafferentation of extraocular muscles. Journal of Neurophysiology **58,** 795-815

Warwick, R. (1955) The so-called nucleus of convergence. Brain **78,** 92-116

Wilson-Pauwles, L., Akesson, E. and Stewart, P. (1988) Oculomotor nerve. In Cranial Nerves pp 25-47 BC Decer Inc. Toronto

Woodlief, N.F. (1980) Initial observations on the ocular microcirculation in man; the anterior segment and extraocular muscles. Archives of Ophthalmology **98,** 1268-1272

Zèlèna, J. & Soukup, T. (1977) The development of Golgi tendon organs. Journal of Neurocytology **6,** 171-194