The structure and innervation of sheep extraocular muscles

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THE STRUCTURE AND INNERVATION OF SHEEP EXTRAOCULAR MUSCLES

VOL. I. TEXT

A thesis presented in candidature for the degree of

Doctor of Philosophy

by

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Durham, October 1974

ACKNOWLEDGEMENTS

I am indebted to Prof. D. Barker for his guidance and constant encouragement throughout the course of this study, and for his valuable criticism of the manuscript. My thanks are also due to Dr. M. J. Stacey for his patient guidance in the use of the electron microscope.

Sheep material was obtained from N. W. Suddes, butchers, Langley Moor, and special appreciation is due to the slaughterman, Mr. F. Barron, for his co-operation.

I am grateful for the skilled technical assistance given by Mr. D. Hutchinson (photographic); Miss Kathleen Flower (preparation and processing of paraffin-embedded material); Miss Susan Grocott (preparation of sections for electron microscopy); and Mrs. M. Spence and Mrs. J. Morley (typing of the manuscript).

Last, but not least, I wish to express my gratitude to my wife, Jill, for her unfailing moral support and her help in preparing the final manuscript.

The investigation was financed by a grant from the Medical Research Council.

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ABSTRACT

The structure and innervation of the extrafusal muscle fibres and the muscle spindles in sheep superior rectus, levator palpebrae superioris, and peroneus brevis muscles are compared.

Superior rectus is organized into three layers: A central core of mainly large-diameter fibres contains three plate-innervated types of 'twitch' fibre, with 7% large-diameter grape-innervated fibres; an orbital rim of small-diameter fibres contains mainly plate-innervated 'twitch' fibres, together with a third of small-diameter grape-innervated fibres; and a thin peripheral patch at either end of the muscle is composed mainly of intermediate-diameter grape-innervated fibres.

Levator palpebrae and peroneus brevis are not layered and they are composed of three plate-innervated types of 'twitch' fibre, the 'slowest' of which are not represented in superior rectus.

The functional significance of the histochemical and ultrastructural characteristics of the fibre types is discussed.

In superior rectus, about 180 spindles are peripherally distributed in the orbital rim and peripheral patch layers, throughout the length of the muscle. The ultrastructure, histochemical profile, and innervation of the nuclear-bag and nuclear-chain fibres are the same as the extrafusal peripheral grape-innervated (G) and plate-

innervated (C) fibre types, respectively. Motor innervation is collateral and segregated with grape innervation distributed to the bag fibres, and plate innervation to the chains.

Levator palpebrae has about 60 evenly-distributed spindles. The bag fibres correspond histochemically and ultrastructurally to the bag fibres of superior rectus, and receive a purely fusimotor grape innervation. The chain fibres correspond to those of superior rectus, and receive a similar plate innervation. Sheep extraocular spindles receive both a primary and secondary sensory innervation.

In peroneus brevis the spindles are composed of typical bag, intermediate bag and nuclear-chain fibres, and these receive an innervation typical of mammalian hindlimb muscle spindles.

PART I - INTRODUCTION

The extraocular muscles, the four rectus and two oblique muscles that bring about movement of the eyeball, are found in all vertebrates from fish to man, although the degree of movement that they can bring about varies greatly. In mammalian species they can produce various types of eye movement ranging from extremely rapid saccades to very slow vergence movements, and in animals with binocular vision these movements must be precisely co-ordinated. As highly-specialised muscles capable of such fine control the eye muscles are of obvious interest in the field of muscle research.

The early histological studies on mammalian eye muscles resulted in the identification of 'terminaisons en grappe' or grape endings (Retzius, 1892) and also, in some species such as man, muscle spindles (Siemmerling, 1888). The first observation was of great significance since it implied that the innervation pattern of mammalian eye muscles was phylogenetically primitive and perhaps more typical of amphibian and reptilian musculature. The second observation was equally important in that it supported the view that eye muscles possessed some form of proprioceptive sense. Subsequent research has usually been concentrated on either the analysis of the extrafusal components of eye muscles and their innervation, or more rarely on the muscle spindles present in some eye muscles. There has been no comprehensive investigation made of muscle spindles in extraocular muscles which also included a study

of the extrafusal components surrounding them.

The initial aim of the present study was to obtain detailed information about one specific sheep extraocular muscle regarding the nature and distribution of the extrafusal plate- and grape-innervated muscle fibre components, and their relation to the muscle spindles. The eye muscles of sheep are particularly suited for study since they are well-supplied with muscle spindles (Cilimbaris, 1910) and have been the subject of a preliminary light-microscope study (see Barker, 1968).

Superior rectus was selected as a representative from the rectus and oblique muscle group and studied using conventional histological techniques, block silver impregnation, histochemical stains, and, as information accumulated, electron microscopy. To give the investigation added significance it was decided to make a similar study of levator palpebrae superioris and also a hindlimb muscle. Levator palpebrae superioris was chosen as a control extraocular muscle from the levator and retractor group that lack an extrafusal grape-innervated component (see chapter 3). Since it overlies the medial edge of superior rectus it is also conveniently removed in the same dissection procedure. Of the limb muscles, only foot muscles were easily available following slaughter of the animal. Choice of a hindlimb muscle was therefore restricted to the distal portion of peroneus brevis. This portion of muscle, like superior rectus and levator palpebrae, was of a convenient

size for histological analysis.

It was hoped that a comparative study of these three sheep muscles would afford an opportunity for significant similarities and differences to emerge and thus broaden the concept of the mammalian muscle spindle and the speculations regarding its motor control.

At the outset of this study there was no published information on the histochemistry or ultrastructure of either the extrafusal muscle fibres of sheep eye muscles or their muscle spindles. During the course of the investigation a brief report on the ultrastructure of the muscle spindles in sheep eye muscles (Scalzi & Price, 1970) confirmed the presence of two types of intrafusal muscle fibre, nuclear-bag and nuclear-chain, and outlined their structural characteristics. Some of the results of this investigation have been published (Barker, Harker, Stacey & Smith, 1972; Barker & Harker, 1972; Harker, 1972a, b).

The findings are presented in three main parts. Part I consists of an introduction to the present study and the materials and methods used. Part II deals with the findings pertaining to the extrafusal muscle fibres, while Part III deals exclusively with the receptors in extraocular muscles. In both Part II and Part III there is a preliminary review of the relevant background information, a description of the results obtained from sheep material, and a discussion of the findings.

MATERIALS AND METHODS

1. Removal of muscles.

Sheep extraocular and foot muscles were obtained from animals that were approximately one year old and reared within normal farm environments. They were brought to slaughter by the usual abattoir methods. After they were stunned and bled the animals were quickly decapitated, enabling immediate access to the eye muscles. Shortly after decapitation of the animal the hindlimb hock joints were broken and the feet removed.

The superior rectus (SR) and levator palpebrae superioris (LP) muscles were exposed by keeping the upper eyelid intact and removing the skin, zygomatic arch and part of the frontal bone to allow superior and lateral access to the orbital cavity. Both SR and LP were clamped across their tendinous insertions onto the globe in order to maintain dorso-ventral orientation. All the extra-ocular muscles were then cut away from the globe and the globe removed. The SR and LP muscles were then dissected towards their common proximal attachment and excised. The arrangement of these muscles *in situ* is shown in figure 1.

The only freely-available fresh hindlimb muscle was peroneus brevis (PB), a short digital extensor. The proximal part of this muscle arises from the dorsal and distal surfaces of the tibial tarsal bone and is lost when the hock joint is cut through.

Thus only the remaining distal portion of PB was used. This portion of muscle inserts on to the tendon of the long extensor muscle in a unipennate manner. The distal portion of PB is shown *in situ*, partially dissected out, in figure 2.

It was possible on two occasions to obtain fresh the distal portion of the 'hough' of sheep which contained several extensors of the foot.

2. Number of muscles used.

In this investigation a total of 23 SR and 19 LP muscles from either the right or left orbits of 17 sheep were used. One SR and one LP muscle were fixed, embedded in paraffin wax and serial transverse sections of both muscles were cut. Seven SR and seven LP muscles were block-impregnated with silver in order to study their innervation. Seven SR and three LP muscles were block stained to demonstrate cholinesterase activity. Four SR and four LP muscles were used to study muscle-fibre histochemistry in frozen transverse sections, and four SR and four LP muscles were processed for electron microscopy (EM).

A total of 10 PB muscles from the right and left hindlimbs of 5 sheep were used. Four were impregnated with silver, four were processed for muscle-fibre histochemistry and two muscles were used for EM study. Two batches of muscles from the 'hough' were used to demonstrate the different histochemical types of muscle fibre found within a 'fast-white' hindlimb muscle.

3. Techniques for wax-embedded material.

One SR and one LP muscle were dissected out, stretched to their approximate resting lengths on pieces of stiff card and sewn into position with thread to prevent excessive shrinkage during fixation. They were immersed in Heidenhahn's Susa fixative for 3 hr, dehydrated in iodized 95% cellusolve for 3 hr, and then in absolute cellosolve for a further 3 hr. The muscles were cleared in a 50/50 mixture of cellosolve and toluene for 6 hr, and then in toluene for 30 min, and embedded in plasticized paraffin wax ('Paramat'). The proximal ends of the muscles were labelled. Serial transverse sections of the whole muscle were cut at 10µm intervals on a Spencer '820' rotary microtome and mounted on albuminised microscope slides.

The sections were stained with Weigert's Iron Haematoxylin and Curtis's Ponceau S substitute for van Gieson (W & VG) according to Humason (1962), rapidly dehydrated through a graded series of ethanols, cleared in xylene and mounted in D.P.X. The W & VG method stains connective tissue pink, muscle fibres yellow and nuclei blue-black. It was thus ideal for the identification of muscle spindle capsules and the classification of intrafusal muscle fibres with regard to their equatorial nucleation as either nuclear-bag (Barker, 1948) or nuclear-chain (Boyd, 1960) fibres. The serial sections were used to map the distribution and length of spindle capsules throughout the lengths of SR and LP.

3.1 Measurements on serially-sectioned material.

Estimations of length were obtained by counting the number of consecutive 10μm thick sections and correcting for shrinkage. The amount of longitudinal shrinkage was assessed by comparing the lengths of the muscles before fixation and after embedding. It was found to be approximately 25%. The spindle-capsule lengths given in the results and shown in figure 189 have been corrected for this amount of shrinkage.

4. Muscle-fibre histochemistry.

4.1 Preparation of fresh-frozen transverse sections.

The muscles were excised within twenty minutes of slaughter of the animal and stretched to their approximate resting lengths on cardboard spills and either frozen complete or cut into origin, belly and insertion portions prior to freezing. In the latter instance excessive contraction during cutting was prevented by using a scalpel that had been dipped in mammalian Ringer solution. The muscles were quick-frozen by direct immersion in iso-pentane cooled to -160^{o}C with liquid nitrogen (Maxwell, Ward & Nairn, 1966). After one minute the end of the spill with the muscle attached was transferred to a deep-freeze and stored at -70^{o}C in a screw-topped container for periods of up to four weeks before further processing (Pearse, 1968). Whole muscles or muscle portions were removed from the spills using pre-cooled scalpel and forceps and were cemented to cryostat chucks with gum tragacanth solidified by immersion in the iso-pentane/liquid nitrogen mixture. The chucks were transferred to a SLEE cryostat and allowed to warm up to -20^{o}C in order to cut frozen transverse sections at 10µm. Consecutive pairs of sections were mounted on uncoated slides and stored within the cryostat. A repeating sequence of histochemical and histological stains was used which usually included W & VG, phosphorylase (P'ase), succinic dehydrogenase (SDH), and myofibrillar adenosine triphosphatase after either alkali (Alk ATPase) or acid (Acid ATPase) pre-incubation.

4.2 Staining methods.

W & VG stain was used in order rapidly to diagnose the state of preservation of the muscle fibres, to identify the cell outlines, and to trace the muscle spindles. Frozen sections were fixed in 5% glutaraldehyde for 3 min, washed in distilled water and stained with W & VG. The sections were washed, rapidly dehydrated, cleared and mounted in D.P.X.

P'ase activity was demonstrated by the method of Takeuchi and Kuriaki (1955), as modified by Eränkö & Palkama (1961). Sections were incubated for 30 min at room temperature. Improved contrast between the different fibre types was obtained by air drying the sections and mounting them under D.P.X. instead of the usual iodine-glycerol. The stained slides were stored in the dark at 5°C for several months without appreciable fading.

SDH activity was demonstrated by the method of Nachlas, Tsou, de Souza, Cheng & Seligman (1957) as modified by Pearse (1961). Nitro blue tetrazolium was used as the electron acceptor at a pH of 7.6. Sections were incubated for 30 min at 37°C.

Myofibrillar or actomyosin adenosine triphosphatase (ATPase) was demonstrated according to the Guth & Samaha (1969; 1970) modifications of the method of Padykula & Herman (1955a). Prior to incubation the sections were pre-fixed in 2% formaldehyde buffered at pH 7.6 then pre-incubated in either alkaline (Alk ATPase)

or acid (Acid ATPase) buffer. For Alk ATPase different pre-incubation media with pH values ranging from 9.8-10.4 were employed. For Acid ATPase a range of different pH values from 4.0-4.6 was used. Following the pre-incubation procedures both Alk ATPase and Acid ATPase-processed sections were incubated for 30-45 min at 37^{o}C.

All incubation media and unstable solutions were freshly prepared immediately before use. Histochemical reactions were absent when substrates were omitted from the incubation media. Batches of slides for any one particular stain were simultaneously removed from the cryostat and processed through the same media in order to give uniformity of staining.

A repeating sequence of enzyme stains (P'ase, SDH, Alk ATPase, Acid ATPase) was applied to batches of serial sections sampled specifically from origin, belly and insertion portions of SR and LP.

Because initial work showed variations in gross structure between the origin, belly and insertion portions of SR, a series of frozen transverse sections were cut at 1mm intervals throughout the lengths of one whole SR and one whole LP muscle and simultaneously processed to demonstrate P'ase activity. With P'ase stain the gross layering pattern in SR is readily apparent (see chapter 6), and by using this one stain on skip-serial sections processed under standardized conditions it was hoped to monitor the variations of layering throughout the lengths of the muscles.

4.3 Comparison of histochemical preparations .

Pairs of consecutive, or nearly adjacent, slides that had been stained to demonstrate different enzymes were examined under two Zeiss GFL microscopes set side by side. Matching fields were orientated and individual muscle fibres were classified with regard to their enzymatic reactions according to the criteria discussed in chapter 4. By replacing one of the slides with the next slide in the series histochemical profiles of individual muscle fibres were built up.

4.4 Measurements on histochemical preparations.

Measurements on fresh frozen material, as well as on teased preparations (see Materials and Methods, 5.1 and 5.2) were made using a x16 Zeiss micrometer eyepiece and a x40 objective calibrated against a stage micrometer slide.

The diameters of the different muscle fibre types were measured on sections stained to demonstrate P'ase activity, and were obtained by taking the mean of two orthogonal diameters. P'ase stain was selected since most of the histochemical fibre types could be distinguished quite easily (see chapter 5.). In SR and LP muscle fibres were measured in fascicles selected from peripheral or central layers of the muscle. Samples were not entirely random since certain infrequently-occuring fibre types were selected to achieve a suitable sample size. The diameter values have not been corrected for the average shrinkage of 2% of the tissue block due to the quenching process (Pearse, 1968) or for the shrinkage

inherent in the phosphorylase staining technique. A comparison of the diameters of individual muscle fibres stained for P'ase against the diameters obtained when the frozen sections are initially fixed, as for W & VG or Alk ATPase, showed that this latter shrinkage factor was in the order of 8% (see e.g. figs.14 & 16).

5. Techniques to demonstrate nerve endings within whole muscles.

Block-staining techniques were used for both silver impregnation and cholinesterase staining and selected portions of material were subsequently teased. Such teased preparations allowed more extensive and accurate tracing of muscle and nerve fibres than is possible by serial reconstruction.

5.1 Silver impregnation.

Preparations of extrafusal nerve endings and whole spindles were made using the modified de Castro silver impregnation method of Barker & Ip (1963) incorporating the improvements suggested by Barker, Stacey & Adal (1970). Silver impregnation was prefered to alternative gold chloride and methylene blue techniques because of its more precise delineation of the nerves and their endings. This is important in an investigation involving study of 'slow' muscle fibres which are supplied by small-diameter nerve fibres terminating in extremely fine and complex endings.

5.11 Barker & Ip silver method.

The application of the Barker & Ip method to the whole SR muscle frequently resulted in excessive staining of the connective tissue, which is particularly extensive in the periphery of the muscle, coupled with poor impregnation of the central fascicles. To overcome these difficulties in SR the perimysium externum was

removed and the muscle teased into dorsal and ventral fascicle bundles during the initial fixation stage. The concentration of ammonia in the next step was also reduced slightly to three drops of ammonia solution (sp.gr. 0.88) per 100ml of 95% alcohol.

5.12 Combined Gladden and Barker & Ip method.

The modified pyridine-silver stain of Gladden (1970) was also used, either in accordance with her schedule, or in combination with Barker & Ip's method as follows:

1. Fix in a mixture of chloral hydrate, 1g; 95% ethanol, 45ml; distilled water, 50ml; conc. nitric acid, 1.25ml, for 5-6 days.
2. Wash in filtered running tap water for 24 hr.
3. Blot and transfer to 100ml of 95% ethanol containing three drops of ammonia solution (sp.gr. 0.88) for 24 hr.
4. Wash for 30 min in distilled water.
5. Leave in full strength pyridine for 24 hr.
6. Wash for 24 hr in distilled water, changed 5-8 times.
7. Place in 2% silver nitrate for 3 days in the dark at 25^{o}C.
8. Reduce in freshly-prepared solution of 2g hydroquinone in 100ml of 20% formic acid.
9. Rinse in distilled water and store in pure glycerol.

All methods, if performed with meticulous care, produced satisfactory results. The muscle fibres, connective tissue and nuclei stained lightly and the intramuscular nerve trunks and their

branches were well impregnated. Generally the fine terminals of the motor endings were distinct, but only in rare cases were the sensory endings within the muscle spindle and the fine terminations of a Golgi tendon organ successfully impregnated.

Muscle fascicles were teased in glycerol using mounted opthalmic needles under a Zeiss Stereomicroscope III equipped for both incident and transmitted light and with a foot-operated focus. The teased specimens were mounted in glycerol on microscope slides and the coverslips were 'ringed' with pitch.

5.13 Measurements of nerve fibre diameters.

In teased, silver preparations of muscle spindles the diameters of the primary and secondary nerve fibres and the fusimotor plate and grape axons were measured at a point approximately 1mm from spindle entry. Because of the variability in the thickness of the myelin sheath, 5 separate measurements were taken of the total internodal diameter of the same internode and the mean calculated. In the cat Stacey (1969) has calculated that in order to convert measurements of myelinated nerves stained with silver, which suffer from severe shrinkage, into their equivalent diameters in fresh material, a factor of 1.41 must be applied. The values obtained in the present study were corrected for shrinkage using Stacey's factor.

5.2 Cholinesterase staining.

Teased cholinesterase preparations used to demonstrate the sub-neural apparatus of extrafusal nerve terminations were obtained by using an adaptation (Barker, Stacey & Adal, 1970) of the 'direct-colouring' thiocholine method of Karnovsky & Roots (1964) that gives results with block-stained material.

Some SR and LP muscles were used fresh, but in most cases the muscles were initially quick frozen as for histochemistry. The muscles were extended to their approximate resting lengths on cardboard spills, immersed in a mixture of iso-pentane and liquid nitrogen at -160°C and stored at -70°C in air-tight containers. They were removed from cold storage and immersed in ice-cold 10% calcium formol still attached to the cardboard spills used in the freezing procedure. As the muscles thawed in the fixative they were sewn onto the card in order to maintain dorso-ventral orientation and also to prevent undue shrinkage.

The optimum fixation time in calcium formol at 4°C was between 4-6hr. Longer fixation resulted in reduced staining of the extrafusal and intrafusal multiterminal grape endings. During the fixation stage the muscles were periodically removed from refrigeration and teased under a Zeiss Stereomicroscope III. The perimysium externum was removed and the muscles were either left *in toto* with the muscle fibre bundles teased apart slightly, or else separated into small fascicle bundles teased as completely as possible from

origin to insertion. These procedures ensured adequate penetration of the fixative and, subsequently, of the incubation medium. Since orientation of the muscles had been carefully maintained, fascicle bundles could be teased from specific layers of the muscle. The whole muscles or fascicle bundles were then washed in distilled water for 5 min and incubated at room temperature. The incubation medium of Karnovsky & Roots (1964) was modified by the use of an acetate buffer at pH 5.0 instead of pH 6.0, and the incorporation of a 50mM instead of a 5mM solution of potassium ferricyanide. Small pieces of muscle were removed from the incubation medium at 5 min intervals for one hour. The optimum incubation time for the staining of extrafusal end-plates was found to be 10-15 min, and for extrafusal multiterminal endings and intrafusal endings it was 45 min. After incubation the muscles were rinsed in distilled water, blotted, and cleared in glycerol for a minimum of 5 days. It was then possible to tease the fascicles into separate muscle fibres by using fine insect-mounting needles. Isolated preparations were mounted in glycerol.

5.21 Pharmacology of the nerve endings.

Muscle fascicle bundles were incubated for 1-2 hr with either acetylthiocholine iodide (ATChI) or butyrylthiocholine iodide (BTChI) as substrates. To demonstrate acetylcholinesterase, fascicles were immersed for 30 min in a 1mM iso-OMPA solution to inhibit non-specific cholinesterases, washed in distilled water for

5 min and then exposed to ATChI substrate. Non-specific cholinesterases were demonstrated by using a 1mM solution of 284C51 to inhibit acetylcholinesterase, before exposure to BTChI substrate.

5.22 Measurements on cholinesterase preparations.

In the case of singly-innervated muscle fibres measurements were made of the fibre itself and also of the diameter of the sub-neural apparatus if it appeared 'en face'. The diameter of the sub-neural apparatus was assessed as the mean of two orthogonal diameters, one of which was the long axis. Muscle-fibre diameter was expressed as the mean of several readings taken near the end-plate region. In the case of multiterminally-innervated muscle fibres the length of muscle fibre innervated by almost continuous chains of cholinesterase-positive droplets (the 'innervation zones'), and the distance between such innervation zones, was measured. Muscle-fibre diameter was expressed as the mean of several measurements taken near to each innervation zone.

6. Techniques for electron microscopy.

The muscles were quickly excised, extended to their approximate resting lengths on pieces of library card and fixed for 15 min at 4°C in 3% glutaraldehyde buffered at pH 7.3 with 0.1M sodium cacodylate (Mercer & Birbeck, 1966). The muscles were then cut into small pieces approximately 5mm long by 1mm square with the long axis parallel to the direction of the muscle fibres, and fixed for a further 1¾ hr. In the case of SR, selection of material was made mainly from the insertion end and the dorsal and ventral fascicles in the belly of the muscle. After fixation the muscle pieces were washed for 2hr in 0.1M sodium cacodylate buffer containing 0.2M sucrose, and post-fixed for 2hr in 1% osmium tetroxide buffered at pH 7.3 with 0.1M sodium cacodylate. The post-fixed material was washed for 1hr in 0.1M sodium cacodylate buffer containing 0.2M sucrose and dehydrated through a graded series of ethanol (15 min each in 50%, 70% and 95%; 60 min in absolute ethanol with three changes). All the solutions up to this stage were maintained at 0-4°C. The remaining steps were carried out at room temperature. The material was processed through two changes of epoxy-propane (15 min); a 50/50 mixture of epoxy-propane and Epon (30 min); a 25/75 mixture of Epoxy-propane and Epon (30 min); and then infiltrated with absolute Epon for eight hours or overnight. Finally, the material was embedded in fresh Epon and hardened for 48 hr at 60°C. The Epon mixture consisted of mixture A (Epon 812, 62ml; dodecenyl succinic anhydride, 100ml) and mixture B (Epon 812, 100ml; methyl nadic anhydride, 89ml) mixed in equal proportions with

2% 2,4,6-tri(dimethylaminomethyl)phenol.

Transverse or longitudinal sections were cut using glass knives on either an LKB Ultrotome or a Reichert Om U2 ultramicrotome. Semi-thin (1-2μm) transverse sections of the muscle blocks were mounted on microscope slides, stained with a 1% solution of toluidine blue in 1% borax and examined under the light microscope. Specific areas of the block were trimmed to enable ultrathin ('silver-gold') sections to be cut. In many cases the block was turned through 90° to obtain longitudinal sections of the same structure seen in transverse section. The ultrathin sections were mounted on either 50-mesh, Formvar-coated copper grids or on uncoated 200-mesh copper grids and were double-stained with uranyl acetate (5 min) and lead citrate (3 min) (Brody, 1959; Reynolds, 1963). Sections were examined in an A.E.I. EM 801 at an accelerating voltage of 60kV.

7. Photography.

Representative areas of light-microscope preparations were chosen on the basis of clarity and photographed with a Zeiss Ultraphot II microscope using Ilford FP-4 film with a green filter to improve the contrast and general clarity of the final print. The Ultraphot was calibrated against a micrometer slide to obtain the precise magnification at which photographs were taken, and the negatives were enlarged by a standard factor of 1.8. Since histochemical muscle-fibre types were distinguished using the original histochemical preparations, no further photographic standardization was employed. In teased preparations, since the detail often extended through several planes of focus, it was often necessary to compose a single montage from several photomicrographs taken at differing focal levels.

PART II - EXTRAFUSAL MUSCLE FIBRES

SECTION A. REVIEW OF BACKGROUND INFORMATION

Chapter 1. Morphology of the rectus and oblique extraocular muscles of mammals.

Several historical reviews of the morphology of extraocular muscles have been made. The most comprehensive include those of Irvine (1936), Hosokawa (1961), Hess (1967; 1970) and Peachey (1968; 1971). The information in this particular chapter is confined to the four rectus and two oblique extraocular muscles of mammals, and excludes the levator palpebrae and retractor bulbi muscles of the orbit. These are discussed in chapter 3.

Because of the complex structural organization of the rectus and oblique muscles the information is dealt with under the three sub-headings of gross structural organization; motor nerves and their terminations; and finally the classification of muscle fibre types.

1.1 Gross structural organization.

Eye muscles are organized into at least two distinct layers that may exhibit a particularly complex three-dimensional architecture due to branching and interconnection of muscle fibres, some of which do not run the full length of the muscle.

The rectus and oblique muscles of several mammalian species including hedgehog, rabbit, cat, dog, sheep and man were first shown by Woolard (1931) and Voss (1936) to consist of a central core of mainly thick fibres, and an orbital rim of thin fibres. These two layers vary in their degree of separation in different mammalian species (Kato, 1938; Cooper & Fillenz, 1955). In man and monkey the two layers are quite distinct; in dog, cat and goat a transition zone of mixed fibre types is present between the two layers; and in rabbit eye muscles the layers are well-separated in some places and ill-defined in others. Other mammalian species with a similar layered organization include the brown rat (Siebeck & Krüger, 1955), mangabey monkey (Cooper & Fillenz, 1955), guinea pig (Hess, 1961a), rhesus monkey (Zenker & Anzenbacher, 1964), and bank vole (Kaczmarski, 1970a).

In histochemical preparations that demonstrate oxidative enzymes, such as succinic dehydrogenase, the peripheral layers generally exhibit a higher level of activity than the central portion of the muscle. Such 'redness' of the peripheral layers is shown in rhesus monkey (Miller, 1967), cat superior rectus (Nemet & Miller, 1968), and in rat, kitten and guinea-pig eye muscles (Asmussen, Kiessling & Wohlrab, 1970; 1971). In the superior rectus muscle of the rhesus monkey (Miller, 1967) the peripheral small-fibre layer constitutes a C-shaped rim around the muscle and is generally absent from the part adjacent to the globe. In the superior oblique muscle of cat (Asmussen et al.,1971) this outer

rim completely surrounds the central core at the distal end of the muscle, becomes 'C-shaped' in the belly, and has a flat, layered appearance at the proximal end.

The layered organization of eye muscles is further complicated by the branching and interconnection of muscle fibres. In the early literature various forms of branched muscle fibres in eye muscle were first reported by Biesiadecki & Herzig (1858), an observation later confirmed by Tergast (1873) in the eye muscles of sheep. Sherrington (1894a) also briefly noted that some muscle fibres in the eye muscles of cat and rhesus monkey are branched 'like the fibres of tongue muscles', and Dogiel (1906) observed fibres in eye muscles that thickened slightly before splitting into two, three or more fine short branches. He also observed 'short' fibres that could arise and terminate throughout the length of an extraocular muscle simply by tapering down to a point, or else splitting as described previously. Later reports also suggest that particular fibre types are restricted to different parts of eye muscles. In the rabbit (Hines, 1931) the origin third of the muscle contains mainly large-diameter muscle fibres, the middle third contains both large- and small-diameter fibres (presumably with the thin fibres located peripherally) and the insertion third is composed of mainly small-diameter fibres. Many of the larger fibres end abruptly in tendons somewhere in the middle third of the muscle and many small fibres taper and end in the insertion third. In the light of all these observations the statement of Lockhardt & Brandt (1938) that in adult eye muscles the fibres run end-to-end is surprising. In the eye muscles of man (Cooper,

Daniel & Whitteridge, 1955; Voss, 1957), the arrangement differs slightly from that of rabbit, although there are differences in the fibre composition throughout the muscle length. In man a considerable peripheral rim of small muscle fibres extends from the belly to the tendon of origin, whereas close to both origin and insertion tendons almost all the fibres are small. The core of the muscle, especially in the belly, contains large muscle fibres.

The recent utilization of cholinesterase staining has shown that some, at least, of the extraocular muscle fibres of cat (Floyd, 1970) and rat (Mayr, 1971) do not run the full length of the muscle, since they form end-to-end junctions with other muscle fibres with a palisade of cholinesterase-positive material interposed between them. These junctions in cat are particularly common in the middle third of the muscles. In rat they are most frequently found in the proximal and distal parts of the peripheral small-fibre layer.

1.2 Motor nerves and their terminations.

There are references early in the literature to the remarkably rich nerve supply to the relatively small extraocular muscles. In sheep (Tergast, 1873) the ratio of nerve to muscle fibres lies within a range of 1:3 to 1:15, whereas in man (Bors, 1925-6) the comparable ratio ranges from 1:4 to 1:7. It is likely that the ratio of muscle fibres to nerve fibres is in fact higher than these figures suggest, since nerve counts may have been made on mixed

nerves that include a considerable sensory component. Also no account is taken of the fact that some muscle fibres do not extend from end to end of the muscle (see 9.1) and that others may be polyneuronally innervated. Nevertheless, as far as the plate-innervated component is concerned, observations of the terminal innervation in silver-stained material often gives the impression of a small motor unit size. For example, in the eye muscles of man (Hirano, 1941) between 7-10 motor end-plates may be derived from one large-diameter nerve fibre.

Cooper and Fillenz (1955) have discussed the possibility that the relative nerve supply to extraocular muscles increases as one progresses along the evolutionary line to man. They estimated the nerve supply of the rat and cat to be roughly equal, that of the mangabey monkey to be higher, and the nerve supply to man and the higher monkeys to be relatively greatest of all.

Following the initial observation in rabbit eye muscles of 'atypical motor endings' (Retzius, 1892) that resembled the 'terminaisons en grappe' found in reptilian muscle (Tschiriew, 1879), many subsequent investigators showed that grape endings as well as plate endings are present in eye muscles (see Hosokawa, 1961). The grape endings were considered by some, like Retzius, to be motor in function, while others presumed that they were sensory. The latter view persisted until Kupfer (1960) demonstrated that both single 'plate' and multiterminal 'grape' motor endings are shown in human extraocular muscle after staining for cholinesterase.

This observation in man was subsequently confirmed (Cheng, 1963; Wolter & O'Keefe, 1963; Wolter, 1964; Dietert, 1965; Zenker & Gruber, 1967; Namba, Nakamura & Grob, 1968a) and plate and grape endings were demonstrated by cholinesterase staining in the eye muscles of guinea pig (Hess, 1961a; Silver, 1963), cynomolgus monkey (Hess, 1962), rhesus monkey (Silver, 1963; Zenker & Anzenbacher, 1964; Mayr, Stockinger & Zenker, 1966; Mayr, Zenker & Gruber 1967) cat (Hess & Pilar, 1963; Floyd, 1970), rabbit (Silver, 1963; Minoda, 1968; Cheng-Minoda, Davidowitz, Liebowitz & Breinin, 1968), goat (Silver, 1963), rat (Teräväinen, 1968a; Namba, Nakamura, Takahashi & Grob, 1968b; Mayr, 1971) and albino mouse (Saltpeter, McHenry & Feng, 1974).

The staining of nerves has shown that in the extraocular muscles of cat and rhesus monkey (Sherrington, 1897), rabbit (Hines, 1931), and man (Cooper & Daniel, 1949), the motor end-plates form a terminal innervation band, either in the middle portion of the muscle (cat and rhesus monkey) or in the origin third (rabbit). The use of cholinesterase staining has confirmed this pattern in man (Cheng, 1963; Dietert, 1965; Namba *et al.*, 1968a), rhesus monkey (Zenker & Anzenbacher, 1964), rat (Namba *et al.*, 1968b; Mayr, 1971), guinea pig (Buckley & Heaton, 1968) and albino mouse (Saltpeter *et al.*, 1974). The grape endings in these animals are scattered throughout the rest of the muscle.

Plate and grape endings both occur in the peripheral and central layers of extraocular muscles. In the guinea pig (Hess, 1961a), grape endings are found on both the thin 'Felderstruktur'

muscle fibres around the periphery and the thicker 'Felderstruktur' fibres of the central core. In rabbit eye muscle (Cheng-Minoda et al.,1968) the distribution of grape endings is described as being predominantly peripheral, but with some grape innervation in the interior of the muscle. In rhesus monkey (Mayr et al.,1966) grape-innervated fibres total about 17% of the fibres in the orbital rim and about 14% of the fibres of the central core. The corresponding figures for rat (Mayr, 1971) are 20% and 10%, respectively.

Plate endings are therefore also found in the orbital rim of thin muscle fibres (Whitteridge, 1960), and in some cases the proportion of plate to grape endings may be as high as 5:1 (Mayr et al.,1966).

In guinea-pig eye muscles Hess (1961a) has shown, by isolating cholinesterase-stained muscle fibres, identifying the type of ending and processing for electron microscopy, that grape endings are distributed to fibres with a 'Felderstruktur' (field-like) appearance (see 1.3) and plate endings to muscle fibres with a 'Fibrillenstruktur' (fibrillar) appearance. Similar observations have since been made for cat (Hess and Pilar, 1963), man (Dietert, 1965) and rhesus monkey (Mayr et al.,1966).

The fine structure of the grape endings in cat extraocular muscle (Cheng & Breinin, 1965; Pilar & Hess, 1966; Hess, 1967) resembles that of the multiterminal endings on frog 'slow' fibres (Page, 1965). The axon terminals are located either in shallow grooves or on the surface of the muscle fibre with only rudimentary

or irregular post-junctional folding. The plate endings in cat correspond in their ultrastructure to the typical motor end-plates of mammalian limb musculature (see for example, Andersson-Cedergren, 1959): the axon terminals are located in synaptic grooves with numerous post-junctional folds. The plate and grape endings of rhesus monkey (Mayr et al., 1966) and rabbit (Cheng-Minoda et al., 1968) correspond in their ultrastructure to the comparable endings in cat eye muscles. In the albino mouse, however, the plate endings on the 'small red' fibres have very few junctional folds (Saltpeter et al., 1974).

There are indications in the literature, however, that two types of grape ending are present in eye muscles, one of which may be restricted to the peripheral small-fibre layer, while the other occurs in the central core of larger-diameter fibres. Firstly, grape endings are located in both peripheral and central muscle layers in the eye muscles of the guinea pig (Hess, 1961a), rhesus monkey (Mayr et al., 1966), rabbit (Cheng-Minoda et al., 1968) and rat (Mayr, 1971). Secondly, two or more forms of grape endings have been described in a number of species, but without reference to their location in the muscle. In rabbit (Cheng-Minoda et al., 1968) there are 'typical' grape endings with numerous, closely-spaced neuromuscular junctions and also other grape endings with sparser and more widely-spaced terminations. In rat (Teräväinen, 1968a) there are extensive 'type 2' and compact 'type 1' endings comparable to those of the rabbit. Since both types of grape ending are sometimes present on the same muscle fibre, Teräväinen suggests

that some fibres may be polyneuronally innervated via two different 'en grappe' systems. Alternatively the two types of grape ending could represent extremes of a wide spectrum of form such as occurs in rhesus monkey (Zenker & Anzenbacher, 1964). Finally, in a recent study of rat eye muscles Mayr (1971) states that the medium-diameter 'Felderstruktur' fibres in the central core of the muscle have 'small' multiple motor junctions that are evenly distributed along the fibre, whereas the small-diameter fibres of the periphery are supplied with 'larger' multiple motor endings.

There is also recent evidence that the grape endings in eye muscles are not structurally identical to the classic 'en grappe' endings of frog. In cholinesterase preparations of rat (Teräväinen, 1968a; Mayr, 1971) and rhesus monkey (Zenker & Anzenbacher, 1964) eye muscles, weakly-developed post-synaptic folds can occur under some of the grape terminals. Mayr (1971) reports that in rat this folding occurs only under the more extensive grape endings in the peripheral layer; the central grape endings are smooth. An ultrastructural analysis of rat eye muscles (Teräväinen, 1967; 1968a, 1968b) had previously indicated that, in contrast to the plate endings that had regular post-synaptic folds, the two types of grape ending (his type 1 and type 2) could both have completely smooth junctions or else show sparse and irregular post-junctional folding. In addition, a small proportion of grape endings in rat (Teräväinen, 1969) possess an axonal protrusion into the muscle cell. Such pseudopodial protrusions of the axon terminal are also illustrated in cat superior rectus muscle

(Cheng & Breinin, 1965) and it seems that close apposition of axoplasmic and sarcoplasmic membranes can occur in these junctions. More recently, Mukuno (1968) has described six types of neuromuscular junction in the eye muscles of man: two types possessed regular junctional folds ; one type had regular but wide junctional folds; and two types showed no junctional folds. The final type exhibited a folded junction under one axon terminal whereas the junctions beneath two neighbouring terminals were smooth. Mukuno suggests that this type of ending may indicate a polyneuronal innervation.

1.3 The classification of muscle-fibre types.

With the light microscope the muscle fibres in extraocular muscles were first distinguished according to their sarcoplasmic content. Knoll (1891) included the eye muscles of the dog in a vast survey of mammalian muscles that contained 'protoplasm-rich' and 'protoplasm-poor' fibres, and Cilimbaris (1910) noted that all possible intermediate forms exist between these two fibre types in sheep eye muscles.

An unusual fibre type, first reported (Thulin, 1914) as a substantial component of the normal extraocular muscles of man and baboon, has subsequently proved to be relatively rare. It displayed the so-called 'Ringbinden' formations that have been observed in pathological muscle (Heidenhain, 1918) where the peripheral fibrils run circularly around the longitudinal muscle fibres. More

recent work (Wohlfart, 1932; 1938; Cooper & Daniel, 1949; Voss, 1957) has confirmed the presence of 'Ringbinden' in the normal eye muscles of man, baboon, dog and cat, but has shown that they comprise only one per cent of the total muscle fibres present (Wohlfart, 1932). It seems likely that they are the consequences of age or a pathologic condition.

In 1938 Wohlfart described 'a'- and 'b'- fibres in the eye muscles of man, calf, dog, cat, rabbit, guinea pig, pig, sheep, macaque monkey and baboon. These 'a'-and 'b'-fibres are probably equivalent, respectively, to the 'Fibrillenstruktur' and 'Felderstruktur' fibres later described by Siebeck & Krüger (1955) in man, rabbit, cat, dog, guinea pig, brown rat and albino mouse.

In the terminology of Krüger (1949) 'Fibrillenstruktur' refers to the fibrillar appearance of a muscle fibre in transverse section with well-demarcated myofibrils separated by abundant sarcoplasm. 'Felderstruktur' refers to the field-like appearance of a muscle fibre in transverse section with poor and irregular delineation of myofibrils by sparse sarcoplasm.

Hess (1961a) first succeeded in identifying 'Fibrillenstruktur' and 'Felderstruktur' fibres under the electron microscope, in the eye muscles of the guinea pig. 'Fibrillenstruktur' fibres were characterised in transverse section by small, discrete myofibrils that are well-delineated by sarcoplasmic reticulum, while in longitudinal section the myofibrils had an orderly arrangement with a

straight Z line. 'Felderstruktur' fibres showed large, irregular myofibrils in transverse section, and in longitudinal section had a jagged Z line. The comparable 'Felderstruktur' fibres in the eye muscles of the cat were initially reported (Pilar & Hess, 1966) to lack transverse tubules and triads, in contrast to the regular and frequent triads of the 'Fibrillenstruktur' fibres, although further investigation (Hess, 1967) showed the presence of an irregular and sparse triad system.

While only two types of muscle fibre, 'Fibrillenstruktur' and 'Felderstruktur' have been distinguished in the eye muscles of guinea pig (Hess, 1961a), man (Dietert, 1965; Brandt & Leeson, 1966; Mukuno, 1967), rabbit (Cheng-Minoda et al.,1968) and the superior oblique muscle of the cat (Hess & Pilar, 1963; Pilar & Hess, 1966; Hess,1967), three types of fibre have been described in the bank vole (Kaczmarski, 1970a) extraocular muscles and in the inferior oblique muscle of the cat (Peachey, 1968), only one of which clearly has a 'Felderstruktur' morphology. In studies of rhesus monkey eye muscles both Mayr et al. (1966) and Cheng & Breinin (1966) have described two types of 'Fibrillenstruktur' and one type of 'Felderstruktur' fibre, while Miller (1967) for the same species reports five fibre types of which only one possesses a 'Felder-struktur' appearance. Mayr (1971) has subsequently described six ultrastructural fibre types in rat eye muscles, two of which show poorly-separated myofibrils ('Felderstruktur type').

The recent increase from two to several ultrastructural fibre types is matched by a similar proliferation of histochemical fibre types. Thus, whereas there are three distinct levels of succinic dehydrogenase activity in rat eye muscles (Nachmias & Padykula, 1958) and two distinct levels in rabbit (Cheng, 1964), the use of batteries of several different histochemical stains has differentiated three major fibre types in the eye muscles of man and baboon (Durston, 1974), five major fibre types in the eye muscles of rhesus monkey (Miller, 1967), and six fibre types in the eye muscles of rat, cat and guinea pig (Asmussen et al.,1970; 1971).

The complexity of eye muscle organization and the numerous different nomenclature systems, especially with regard to histochemistry, hinders easy comparison of the literature. It is not surprising, therefore, that there are points of controversy concerning the structural characteristics of the fibre types present in eye muscles. Some of these points may well be due to the problem of interpretation of information rather than specific species differences.

A point in question is the discrepancy over whether the grape-innervated muscle fibres in eye muscles are characterised by a lack of an M line as are the 'slow' fibres of frog (Peachey & Huxley, 1962; Page, 1965). Thus while cat 'Felderstruktur' fibres are reported to posses an M line (Pilar & Hess, 1966; Hess, 1967; Peachey, 1968), as do the 'Fibrillenstruktur' fibres, the eye

muscles of the rhesus monkey contain both thin 'Felderstruktur' fibres (presumably peripheral) without an M line (Mayr et al., 1966) and thick, central 'Felderstruktur' fibres with a poorly-developed M line (Miller, 1967). An attempt to clarify the issue by analysing the ultrastructure of isolated, cholinesterase-stained muscle fibres from rabbit eye muscles (Cheng-Minoda et al.,1968) showed that the muscle fibres with profuse grape innervation lack an M line, although the fine structure of other fibres with 'less-frequent' grape innervation was not assessed. Recently it has been found that both the central and peripheral 'Felderstruktur' fibres in the eye muscles of the rat (Mayr, 1971) and the bank vole (Kaczmarski, 1970a) lack an M line, as do the grape-innervated fibres in the extraocular muscles of the albino mouse (Saltpeter et al.,1974).

Although it is likely that there is some species variation with regard to presence or absence of an M line in 'slow' or 'fast' eye muscle fibres, it is also possible that confusion has arisen because of the presence of two types of grape-innervated fibres in eye muscles, each with different location and ultrastructural characteristics (Peachey, 1968; 1971). In cat eye muscles, Peachey (1966; 1970) proposes that one grape-innervated fibre type is peripherally-located, small in diameter, rich in mitochondria, and displays a morphology characteristic of 'fast' fibres; while a second type is centrally-located, larger in diameter, poorer in mitochondria and exhibits a 'Felderstruktur' morphology. Both types posses an M line.

Chapter 2. Physiology of the rectus and oblique extraocular muscles of mammals.

The neurophysiology of eye movements has been comprehensively reviewed at regular intervals over the past two decades (Cooper, Daniel & Whitteridge, 1955; Bach-y-Rita, 1959; 1971; Whitteridge, 1960; Peachey, 1968; Hess, 1970; Breinin, 1971). The following constitutes a brief survey of current opinions with regard to the extrafusal muscle fibres.

The numerous fibre types distinguished in histochemical and ultrastructural studies are not matched by a comparable variety of physiological and pharmacological responses, and generally speaking only 'slow' and 'fast' components have been identified in mammalian eye muscles.

Some responses of cat eye muscles, such as the extremely rapid twitch of 20-28msec duration, the short refractory period, and high tetanus fusion frequency of between 300 and 450 cycles/sec (Cooper & Eccles, 1930; Brown & Harvey, 1941; Bach-y-Rita & Ito, 1966a) appear to be based on a particularly fast-acting component. Other responses, such as the prolonged contracture on exposure to cholinergic compounds (Duke-Elder & Duke-Elder, 1930; Brown & Harvey, 1941; Katz & Eakins, 1967; Eakins & Katz, 1971) or to low-frequency stimulation of the motor nerve (Hess & Pilar, 1963; Bach-y-Rita & Ito, 1966a) appear based on a 'slow' component that may be comparable to the tonus fibres in the frog iliofibularis muscle.

The use of intracellular microelectrode recording techniques has distinguished only two major types of muscle fibre in the eye muscles of rabbit (Matyushkin, 1961; Ozawa, 1964; 1965) and cat (Matyushkin, 1963; Hess & Pilar, 1963; Pilar, 1967). In addition to 'fast' fibres that respond to a single stimulus with an all-or-none, propagated action potential and a brief, total contraction and relaxation of the muscle fibre, these authors distinguish 'slow' fibres that respond only to repetitive stimulation with multiple, non-propagated small junctional potentials and give sustained, local, graded contractions. Such slow fibres in extra-ocular muscles may be compared with amphibian slow fibres first physiologically characterized in frog (Tasaki & Mizutani, 1944; Kuffler & Vaughan-Williams, 1953a, b).

The physiological evidence indicates that the slow fibres of cat and rabbit eye muscles are supplied by small-diameter nerves (Matyushkin, 1963a), and that their junctional potentials arise from multiple sites within the fibres (Matyushkin, 1963a; Hess & Pilar, 1963; Pilar, 1967) and are polyneuronal in origin (Pilar, 1967).

A central point of controversy has arisen because Bach-y-Rita & Ito (1966a) have reported that although slow and fast fibres in the eye muscles of the cat may be distinguished by several physiological parameters, the slow fibres are also capable of producing propagated action potentials when suitably stimulated. They attribute impalement damage as the cause of failure of some of their slow

fibres to propagate. A subsequent combined physiological/morphological study (Alvarado, Adrian, Bach-y-Rita & Peachey quoted in Bach-y-Rita, 1971) has shown that some of the slow fibres in the peripheral layer of cat eye muscles that are capable of producing propagated action potentials possess multiterminal innervation. Bach-y-Rita (1971) has thus stressed the existence of "slow multi-innervated twitch" fibres in the eye muscles of cat.

Further work by Matyushkin & Drabkina (1970) on the superior oblique muscle of rabbit has confirmed the absence of spike action potentials in the slow fibres of this species. These authors also give a more detailed account of the two types of excitatory post-synaptic potential in rabbit slow fibres: a faster ('spike-like') and a slower type, first described by Matyushkin (1963b). Since in many cases the two types of potential are generated in the same slow fibre (in neighbouring synapses) the possibility exists that there are several types of motoneuron supplying extraocular slow fibres.

The simultaneous recording of intracellular and electromyogram (EMG) potentials in rabbit eye muscles (Ozawa, Cheng-Minoda, Davidowitz & Breinin, 1969) has shown monophasic EMG potentials that are synchronous with the spontaneous junctional potentials thought to occur only in slow fibres (Matyushkin, 1961; Ozawa, 1964; 1965) and that are clearly different from EMG spike potentials. The monophasic EMG potentials closely resemble the extracellular potentials of long duration obtained by Matyushkin (1964) and Nemet

& Miller (1968) that were interpreted by them as arising in slow fibres.

Of 53 fibres showing spontaneous, slow, multi-focus potentials, Ozawa et al. (1968) successfully injected 8 with marker dye and showed them to be multiterminally innervated by staining to demonstrate cholinesterase. None of the 53 fibres could be stimulated to produce action potentials, but overshoot spikes were obtained in 25 additional 'silent' fibres, 2 of which were multiply- and 23 of which were singly-innervated. The possibility thus still remains that in rabbit and cat extraocular muscles two multiterminally-innervated slow systems exist, one of which is relatively common and exhibits monophasic EMG potentials, spontaneous multi-focus potentials and is unable to propagate action potentials, and another system that is less common and which is able to produce action potentials.

A correlation of the activity of slow and fast populations of axons in the VI nerve with the slow and fast phases of vestibular nystagmus (Yamanaka & Bach-y-Rita, 1968) has further shown that while slow axons are active mainly during tonic movements and fast axons are active mainly during phasic movements, each type of axon contributes towards part of each type of eye movement. The physiological basis for the fast and slow movements of eye muscles, initially proposed by Hess & Pilar (1963) to be a simple, dual, functional mechanism of fast and slow muscle fibres, appears to be more complex in organization, and the "duality concept" is now in need of re-appraisal (Breinin, 1971).

Chapter 3. The retractor bulbi and levator palpebrae muscles of mammals.

In addition to the four rectus and two oblique extraocular muscles that suspend and rotate the eyeball there may also be present within the orbit two other associated muscles, the retractor bulbi and levator palpebrae, that function to retract the globe and raise the upper eyelid, respectively. Both of these muscles exhibit a less complex type of organization than that shown by the rectus and oblique group and they both appear to be composed entirely of plate-innervated 'twitch' muscle fibres.

3.1 Retractor bulbi.

The retractor bulbi muscle of the cat lacks the peripheral 'red' layer of small-diameter fibres that is characteristic of the rectus and oblique muscles, and is composed of only three histochemical fibre types (Asmussen, Kiessling & Wohlrab, 1971). These correspond to the 'Fibrillenstruktur' fibre types present in the central core of the rectus and oblique muscles. The muscle fibres consequently all possess a fairly uniform 'fast-twitch' ultrastructure (Alvarado, Steinacker & Bach-y-Rita, 1967) and the muscle does not exhibit contracture on the administration of succinylcholine either in the *in vivo* (Bach-y-Rita & Ito, 1965) or *in vitro* preparations (Bach-y-Rita, Levy & Steinacker, 1967). The electrical (Bach-y-Rita & Ito, 1965) and mechanical

(Steinacker & Bach-y-Rita, 1968a) properties are intermediate between the 'fast' (twitch) and 'slow' ("multi-innervated twitch") muscle fibres of the cat inferior oblique (see chapter 2). The term motor end-plate was first used by Krause (1863) to describe the compact type of neuromuscular junction that he observed in the retractor bulbi muscle of the cat and it has been presumed that the extrafusal muscle fibres are in fact all plate-innervated. Even so, there are reports (Hosokawa, 1951) of 'grape-like' endings in the retractor bulbi muscles of both the cat and whale. The ratio of nerve fibres to muscle fibres in cat retractor bulbi, estimated to be approximately 1:50 (Steinacker & Bach-y-Rita, 1968b), appears to be lower than in the rectus and oblique muscles. It has, however, become apparent that the muscle fibres do not run the full length of the muscle (Bach-y-Rita, 1971).

3.2 Levator palpebrae.

The levator palpebrae muscle of several mammalian species shows no apparent layered organisation (Kato, 1938). In the rhesus monkey it does not contain muscle fibres of 'Felderstruktur' appearance (Miller, 1967), and in the levator palpebrae muscles of man (Dietert, 1965; Namba et al.,1968a) and rat (Namba et al.,1968b) there are only plate endings present. In the baboon and man (Durston, 1974) three histochemical types of muscle fibre are present that are also represented in the rectus and oblique muscles. The majority of the fibres (75-80%) are of the 'coarse' type and show high oxidative and Alk ATPase activity and moderate glycolytic activity (= C-type histochemical profile. See chapter 4).

Chapter 4. Histochemical fibre types in mammalian muscle: nomenclature and sources of variation.

Although the current weight of histochemical evidence points to the existence of three kinds of fast muscle fibres in mammalian skeletal muscles, there is at present no universally accepted system of nomenclature. Fibre types have been variously termed as 'white', 'intermediate' and 'red' (Ogata & Mori, 1964; Edgerton & Simpson, 1969); A, B and C (Stein and Padykula, 1962; Henneman & Olson, 1965; Yellin, 1969b) and α , β and $\alpha\beta$ (Guth & Samaha, 1969; Samaha, Guth & Albers, 1970). It has become increasingly apparent that, in order to obtain a meaningful histochemical profile for a particular muscle fibre, the selection of histochemical stains should include at least one oxidative enzyme such as SDH; one glycolytic enzyme such as phosphorylase; and also actomyosin ATPase as an indicator of intrinsic speed of contraction. Davies & Gunn (1972) have recently shown that three such histochemical stains enables adequate characterization of individual muscle fibres from the diaphragm of several species.

In the present study SDH, P'ase and actomyosin ATPase were selected as the major histochemical stains and it was decided to adopt the A, B & C nomenclature system after the manner of Stein & Padykula (1962) and Yellin (1969b). Those fibres with low oxidative and high glycolytic activity are designated as A fibres; those fibres with high oxidative and low glycolytic activity as B fibres; and those fibres with high oxidative and glycolytic activity

as C fibres. For simplification all 'intermediate' activities are regarded as 'high'. In the majority of sheep fibres studied the activity of actomyosin ATPase corresponded roughly to that of P'ase and can be deduced from the A, B or C nomenclature as high, low or high, respectively. In peroneus brevis, however, one of the three fibre types present has a low actomyosin ATPase activity and a high P'ase activity. Since SDH activity is high this fibre is termed a 'modified' C type.

The adopted nomenclature system describes histochemical fibre types commonly found in mammalian skeletal muscle that is composed entirely of plate-innervated muscle fibres. In the rectus and oblique eye muscles, additional fibre types are present in which the activity of both oxidative and glycolytic enzymes is low (Miller, 1967; Asmussen, Kiessling & Wohlrab, 1970; 1971). Since the evidence in sheep suggests that such fibres receive a multiterminal grape innervation, the histochemical profile has been designated as type G (Barker, Harker, Stacey & Smith, 1972).

It has been seen fit to classify the muscle fibres into discrete fibre types rather than consider them as a continuum (see e.g. Romanul, 1964) in order to provide a working basis for future physiological investigations, for example of the characteristics of motor units. In skeletal muscles, although individual fibres maintain uniform histochemical characteristics along their length (Farrell & Fedde, 1969), there are sources of variation in the proportion of histochemical fibre types. These include sex and stock differences (Vaughan, Aziz-Ullah, Goldspink & Newell, 1974) and

changes due to exercise (Edgerton, Gerchman & Carrow, 1969; Kowalski, Gordon, Martinez & Adamek, 1969; Campbell, Onan, Thomas, Weirich, Will, Cassens & Briskey, 1971). In the case of extraocular muscles the functional demands and the load do not vary to any great extent once growth and development is complete. It might be expected, therefore, that the proportions of fibre types in extraocular muscles would remain fairly constant. The sheep used in the present study were of mixed sex and from different stock and farm environments, and would be subject to this variability.

SECTION B. RESULTS

Chapter 5. Intensity and distribution of histological and histochemical stains.

5.1 Weigert and van Gieson.

This stains connective tissue pink, muscle fibres yellow and nuclei blue-black. In addition, particularly in well-stained frozen sections a pattern of scattered and intensely-stained granules is exhibited that corresponds to the pattern of diformazan granules produced by SDH (see e.g. figs.19 & 20). Such histological sections thus exhibited a checkerboard appearance more characteristic of histochemical stains, and they were useful for fibre typing. The precise, punctate pattern of granules obtained with W & VG most closely resembles the pattern observed in EM semi-thin sections stained with toluidine blue (figs.34-39) and is probably also due to the staining of mitochondria.

5.2 Phosphorylase.

With P'ase fibres varied in their staining reaction from an overall blue-grey network to a dark or lighter brown, to a golden yellow that matched the colour given to sections incubated without a substrate. For the purposes of classification (see chapter 4) blue-grey and dark-brown colourations were regarded as high activity, light-brown and yellow colourations were regarded as low activity. In less diffusely-stained sections the stain is visible at

high power as a network localised in the sarcoplasm around the myofibrils. Since glycogen is the major source of energy for muscular contraction in the absence of oxygen, and phosphorylation is the first step in glycolysis, phosphorylase activity reflects the capacity of a given fibre to contract anaerobically.

5.3 Succinic dehydrogenase.

The sites of activity of SDH were defined by deposits of blue-purple diformazan granules scattered in the sarcoplasm of intrafusal and extrafusal muscle fibres. Variation in the size, number and distribution of diformazan granules enables several muscle fibre types to be distinguished. In order of increasing gross-staining intensity these types comprise clear; white; pale; intermediate; and red SDH patterns. Clear fibres have a fine reticulate net with only few small granules; white fibres show relatively few small granules that tend to form subsarcolemmal accumulations; pale fibres have many small granules distributed in a uniform network; intermediate fibres have subsarcolemmal concentrations of large particles; red fibres possess a uniformly-distributed network of large particles. For the purposes of fibre-typing, red and intermediate fibres were regarded as high activity, and clear, white and pale fibres were regarded as low activity. Since SDH is a mitochondrion-bound enzyme (Roodyn, 1967) the diformazan granules are usually attributed to the mitochondria. It is assumed, since mitochondrial density and oxidative capacity of a given muscle are directly related (Paul & Sperling, 1952), that SDH activity reflects the capacity of a given fibre to contract aerobically.

5.4 Myosin ATPase.

Incubation for ATPase at pH 9.4 after formaldehyde fixation and alkali pre-incubation (Alk ATPase) enabled three major types of muscle fibre to be distinguished: high-activity fibres had a black reaction product and were thus alkali-stabile/formaldehyde resistant; intermediate-activity fibres had a grey reaction product and were thus alkali-stabile/formaldehyde-sensitive; low-activity fibres were white and were thus alkali-labile. Black and grey fibres were considered as high, and white fibres as low for fibre classification. Within a transverse section of a muscle fibre the reaction product is localised on the myofibrils, which thus appear as a punctate network surrounded by relatively clear sarcoplasm (see e.g. fig.16). The correlation of histochemical and biochemical data by Guth & Samaha (1969) suggests that the technique used is relatively specific for actomyosin ATPase which is most active in the alkali range (Padykula & Herman, 1955b; Padykula & Gauthier, 1963). However, since capillary endothelia are also intensely stained by reaction product, (see e.g. figs.26 & 29) the technique is not totally specific for the myofibrils, and other phosphatases may contribute. The activity of myosin ATPase for a particular muscle has been shown to be directly proportional to the intrinsic speed of contraction of that muscle (Bárány, 1967). Burke et al. (1971) in a combined physiological and histochemical study have shown that muscle fibres that exhibit high myosin ATPase activity have fast twitch contraction times, while fibres with low myosin ATPase activity have relatively slow twitch contraction times. Myosin

ATPase activity may therefore be taken as an indicator of the intrinsic speed of contraction of individual muscle fibres.

Incubation for ATPase at pH 9.4 after pre-incubation in acid (Acid ATPase) reversed the staining pattern obtained with Alk ATPase, similar to the EDTA effect noted by Drews & Engel (1966). Since the reaction product within the cell is localised in the intermyofibrillary network (Guth & Yellin, 1971), and capillary endothelia and nuclei are also stained, the technique probably demonstrates intermyofibrillar ATPases and some non-specific phosphatases.

Chapter 6. Layer organization in superior rectus.

A transverse section through the belly of SR stained with W & VG shows the two distinct layers seen by early histologists such as Kato (1938) and Voss (1935). A central core layer of mainly large-diameter muscle fibres lies adjacent to the eyeball, and is covered dorsally and laterally by an orbital rim layer of small-diameter muscle fibres that constitute a C-shaped rim to the muscle (see fig.3). The orbital rim layer contains a richer supply of blood capillaries and more abundant connective tissue than the central core layer, and there is a marked separation of the two layers in the belly of the muscle in a region just anterior to the point of nerve entry. Here, nerve trunks and blood vessels fan out across the muscle from the lateral to medial side, and they tend to lie between the two layers. At the origin and insertion ends of the muscle, however, the separation of orbital rim and central core layers is less distinct with a more gradual transition from the outer small-cell layer to the inner large-cell layer. Also present at either end of the muscle is an additional peripheral layer composed of medium to large-diameter muscle fibres that are characteristically separated from each other by extensive connective tissue. These 'peripheral patch' layers are distributed around the dorsal and lateral periphery of the orbital rim layer in the proximal and distal portions of the muscle.

The use of histochemical stains enable the layers to be distinguished more clearly (figs.4-13). At low magnification the central core layer is characterised by a mosaic of large-diameter

muscle fibres which show a G-type (Barker, Harker, Stacey & Smith, 1972) histochemical profile. They are picked out with both P'ase and Alk ATPase as fibres of extremely low activity that show clearly against a generally high background in the central core, and with Acid ATPase (fig.8) as fibres of very high activity against a very low background. The orbital rim layer also contains fibres with a G-type histochemical profile, but since they are not quite as low in P'ase activity and are not as large in diameter their mosaic is by no means as striking. With P'ase, Alk ATPase and SDH the orbital rim layer shows the greatest overall staining intensity. By comparison, the peripheral patch layer shows the lowest overall staining intensity with all these histochemical stains, and the central core is intermediate in gross activity. Since the stains do not show the abundant connective tissue surrounding the peripheral patch fibres these layers possess a distinctive 'loosely-packed' appearance (figs.5, 10 & 12).

The existence of a peripheral patch layer in addition to the orbital rim and central core layers is not recorded for the extraocular muscles of other mammalian species. However, in rabbit lateral rectus muscle (Asmussen et al., 1971) a comparable peripheral layer is evident in an illustration (their fig.1) that shows SDH activity, although this layer is not distinguished in the text. In other classes of vertebrates a triple-layered organization is present in the superior rectus and superior oblique muscles of the lizard (Kaczmarski, 1969), and in the rectus and oblique muscles of the gudgeon (Kordylewski, 1974) and tree sparrow (Kaczmarski, 1970).

6.1 Variation of layered organization throughout muscle length.

The composition, thickness and distribution of the three muscle-fibre layers found in SR varies thoughout the length of the muscle (see figs.3-13). In the single SR muscle, from which frozen transverse sections were taken at one mm intervals and stained with P'ase, the central core layer was the only one consistently present throughout. In fact, at the extreme origin end of SR only the central core layer is present over the distance that the branch of the oculomotor nerve enters the lateral border of SR near the origin and travels anteriorly without appreciable branching. In the case of the SR muscle examined, which had a total, slightly-contracted length of 32mm, this distance was approximately 3mm (fig.4). The central core layer increases rapidly in depth and width to attain a maximum volume at about 6mm from the origin, and this volume is maintained up to about 20mm. Thereafter the depth of the layer gradually decreases towards the tendinous insertion (figs.10 & 11).

The orbital rim and proximal peripheral patch layers are first detectable at about 4mm from the origin of SR, at the point where the oculomotor nerve branch first begins to divide (fig.5). The orbital rim is initially only a fascicle or two deep and it only intermittently forms a C-shaped rim. The peripheral patch layer overlies the orbital rim dorsally and laterally and is also located around the ventral portion of the orbital rim layer where it lies adjacent to the eyeball. Here a "ventral concentration" of peripheral patch fibres is formed that is relatively close to the fibres of the central core (figs.5 & 6). The proximal peripheral patch

layer in the SR examined extended from about 4mm from the origin to about 11.0mm, the ventral concentrations being the longest of the detectable patch layers.

The orbital rim layer increases in depth to reach maximum volume between about 10mm and 20mm from the origin. In the belly of SR the deep orbital rim appears to be graded into sub-layers with each successive more peripheral sub-layer composed of fibres of increasingly smaller diameter (fig.13). It is in this region of SR that the muscle fibres of extremely small diameter are found. Towards the insertion end of SR the orbital rim layer becomes progressively thinner, so that at 25mm from the origin it is little over one fascicle deep (figs.10 & 12), and about 4mm from the insertion the orbital rim finally disappears (fig.11). The distal peripheral patch layer, which extends from about 18.5mm from the origin to the insertion end, thus directly overlies the central core layer at the extreme insertion end of SR. This peripheral patch layer is more extensive than that found at the proximal end of SR and it also lacks the ventral concentrations of patch fibres seen in the former. Even so, although the layer appears thicker due to the loose arrangement of the muscle fibres, the distal peripheral patch layer is only several cells thick at its deepest point (fig.12).

The muscle spindles are mainly peripherally distributed (see chapter 16): either within the orbital rim in the belly of the muscle or at the junction between the orbital rim and peripheral patch layers at the proximal and distal ends.

The variation of layered organization throughout the length of sheep SR is more complex than that described for rabbit (Hines, 1931;1.1) or man (Cooper et al., 1955; Voss, 1957; 1.1). Whereas the central core remains a constant feature, the orbital rim is absent from the extreme ends of the muscle, and the peripheral patch layer is absent from the belly. Contrary to the findings for rabbit and man, large-diameter muscle fibres are present at both ends of sheep SR.

These findings emphasize the need to sample throughout the whole length of a particular extraocular muscle to obtain a complete picture. Detailed comparisons are possible between species only if the approximate level of any transverse section is specified.

Subsequent investigation of SR was concentrated on the extreme insertion end of the muscle and on the belly. These regions are both simplified in their layering in that only two major layers are present: at the insertion end the peripheral patch layer overlies the central core, while in the belly the central core is overlain by the orbital rim.

Chapter 7. Histochemical fibre types in superior rectus.

Of the three muscle-fibre layers in SR only the central core displays a 'chequer-board' appearance characteristic of skeletal muscle histochemical preparations. The orbital rim and peripheral patch layers are more 'homogeneous' in appearance. Nevertheless, each of the layers is in fact heterogeneous in terms of types of muscle fibre present (see Table I for summary).

7.1 Central core layer.

The central core layer contains four major types of muscle fibre, distinguishable on the basis of differences in diameter and histochemical profile (figs.14-17).

(i) The largest diameter fibres (mean = 31.9μm) found in the central core layer form a characteristic mosaic of fibres that is particularly striking at low magnification and which possess a type G histochemical profile (chapter 4). With P'ase these large G fibres are the lowest reacting fibres in the muscle and are bright yellow in their background colour with only very faint light brown streaks. With SDH the pattern of staining is classified as 'clear' which is also the lowest level of SDH activity found in the muscle. The small, sparse formazan particles are arranged in an open network of fine dots and streaks without any appreciable subsarcolemmal activity. This suggests that the large G fibres possess only few small-diameter mitochondria interspersed between a high concentration of myofibrillar material. The large G fibres show minimal activity for Alk ATPase (pre-incubation at pH 10.2) with the myofibrils

Table I. A summary of the light-microscopic characteristics of the various muscle fibre types in each layer of sheep superior rectus muscle.

Fibre types	CENTRAL CORE LAYER				ORBITAL RIM LAYER			PERIPHERAL PATCH	
	Large G	Large A	Inter. C	Small C	Small G	Inter. C	Small C	Inter. G	Small C
Mean diam. (μm)	31.9	28.9	23.0	17.3	13.4	25.6	18.9	24.4	18.2
P'ase	Low	High	High	High	Low	High	High	Low	High
SDH	Low	Low	High	High	Low	High	High	Low	High
Alk ATPase	Low	High	High	High	Low	High	High	Low	High
Acid ATPase	High	Low	Low	Low	High	Low	Low	High	Low
Fibrillar pattern†	Fe	Fi	Fi	Fi	Fe?	Fi?	Fi?	Fe	Fi?
Innervation	grape	plate	plate	plate	grape	plate	plate	grape	plate
% total fibres within layer	7	45	48		34	34	32	65 - 95	5 - 35

† Fe., Felderstruktur
Fi., Fibrillenstruktur.

appearing white. With Acid ATPase (pre-incubation at pH 4.35) the staining pattern is reversed to give an intense black 'wash', which has no clear localisation. The large G fibres constitute about 7% of the total fibres within the central core layer.

(ii) The second largest fibres in the central core layer (mean = 28.9μm diameter) possess a type A histochemical profile. The activity of these large A fibres with P'ase is the highest in the muscle typically giving a blue-grey colouration that is probably indicative of newly-formed glycogen (Takeuchi & Kuriaki, 1955). With SDH they give a 'white' pattern of formazan particles consisting of fine granules arranged in a fairly loose network around the periphery of the muscle fibre, leaving the centre comparatively clear. The fine streaks characteristic of the large G fibre are not shown, although the overall staining intensity of the large A fibre with SDH is only slightly higher than that of the large G fibre. Both of these fibres are considered low with SDH. The large A fibres show high activity with Alk ATPase, the black reaction product being localised on the myofibrils, while with Acid ATPase the reaction is minimal. The high reaction with Alk ATPase with little of the fibre being unstained suggests a high concentration of myofibrillar material. The large A fibres are especially frequent around the periphery of the fascicles in the central core, and they constitute about 45% of the fibres of this layer.

The two other fibre types of the central core both possess a type C histochemical profile and as such they both exhibit moderate or high levels of activity for P'ase, SDH and Alk ATPase, whereas

Acid ATPase activity is low.

(iii) The larger of these two fibre types, the intermediate C fibre, is of intermediate diameter (mean = 23.0μm) and with P'ase and Alk ATPase shows similar high levels of activity to those shown by the large A fibres. However, with P'ase the reaction product is dark brown in the intermediate C fibres as compared with the blue-grey of the large A fibres. This brown colour probably indicates the staining of native glycogen (Takeuchi & Kuriaki, 1955), and in terms of intensity it often appears denser than the blue-grey colour of large A fibres. The reaction with SDH serves to differentiate the intermediate C fibre from the large A fibre. The diformazan particles are large and tend to form deep subsarcolemmal accumulations leaving the centre of the fibre relatively clear. The fibres thus have a distinct border of high SDH activity with lower activity in the interior of the fibre. They might be expected to contain larger and more numerous mitochondria than either the large G or large A fibre types. With Acid ATPase the intermediate C fibre is not entirely negative. In contrast to the large A fibre types some slight residual intermyofibrillar activity is exhibited.

(iv) The smallest-diameter fibre type in the central core layer is the small C fibre (mean diameter = 17.3μm). With P'ase these fibres exhibit a similar dark brown colour to the intermediate C fibre types and with SDH they are also highly reactive. The large formazan particles in the small C fibres are, however, scattered evenly throughout a transverse section of the fibre with no apparent

'rim' of high activity. With Alk ATPase the small C fibres exhibit intermediate activity with a grey colouration of the myofibrils. They are thus more formaldehyde-sensitive than either the large A or intermediate C fibre types. A high proportion of the small C fibre in transverse section remains unstained with Alk ATPase. These scattered unstained areas presumably correspond to mitochondrial accumulations. The small C fibres give a low to intermediate pattern of staining with Acid ATPase. Like the intermediate C fibres, the small C fibres might be expected to contain numerous large mitochondria and abundant stored particles of glycogen. Together, the intermediate C and small C fibre types form about 48% of the total number of fibres in the central core layer.

7.2 Orbital rim layer.

In the belly of SR the orbital rim layer contains three major fibre types in roughly equal proportions. Two of these types correspond in histochemical profile and relative diameter to the intermediate C and small C fibre types of the central core, while the third type is of very small diameter and exhibits a G-type histochemical profile (figs.18-21).

(i) The intermediate C fibre type of the orbital rim exhibits subsarcolemmal accumulations of large diformazan particles with SDH, stains dark brown with P'ase, grey-black with Alk ATPase, and shows faint residual activity with Acid ATPase.

(ii) The small C fibre type shows intense staining with SDH, with large diformazan particles scattered throughout the

transverse section, stains dark brown with P'ase, grey-black with Alk ATPase, and shows a reversal pattern of low activity with Acid ATPase. These two fibre types are largely distinguishable by their SDH reactions, which tend to be of a higher overall activity in the orbital rim than in the central core, and by their diameters. In the more peripheral fascicles of the orbital rim the diameters of the C fibre types is reduced and the distinction between them with SDH is much less marked. In such peripheral fascicles the C fibre types form more of a continuous spectrum.

(iii) The third fibre type in the orbital rim exhibits a G-type histochemical profile and is the smallest-diameter fibre type in the muscle (mean = 13.4μm). In the middle third of the muscle these fibre types can be remarkably small in diameter (figs. 18-21) but towards the origin or insertion ends of the muscle they are larger (figs.22-24). With P'ase these small G fibres appear light brown or fawn in colouration and are of relatively low activity when compared to the surrounding intensely-reactive C fibre types (figs.18 & 22). With SDH the pattern of staining is classified as 'pale' with many small diformazan particles and streaks scattered throughout the fibre in a uniform network. Again these fibres appear of relatively low activity when compared to neighbouring C type fibres (figs.19 & 23). The small G fibres show minimal activity for Alk ATPase and with Acid ATPase this reaction is reversed to give an intense black (figs.21 & 24). Compared to the large G fibre of the central core, the small G fibre of the orbital rim shows slightly higher P'ase and SDH activity, albeit still relatively low.

Within the insertion third of SR the orbital rim layer progressively reduces in thickness towards the insertion. Here, in addition to the intermediate C, small C and small G fibre types found in the orbital rim of the belly, are found a proportion of large A fibres similar to those of the central core. These large A fibres are most apparent in sections that have been slightly over-incubated for SDH. The intermediate and small C fibres are intensely stained while the large A and small G fibres remain relatively low (fig.23). With P'ase the large A fibres react to give a blue-grey colouration, while the C fibre types give an intense dark brown, and the small G fibres exhibit a light brown, relatively pale colouration.

In the superficial part of the orbital rim layer in rat extraocular muscle a unique fibre type has been described (Yellin, 1969a) that exhibits high activity for both Alk ATPase and Acid ATPase. Yellin postulates that such fibres may possess a dual innervation. Although an extensive range of pH values was employed in the pre-incubation stages of the actomyosin ATPase method (Part I, 4.2) a comparable dual activity was not observed in fibres from either the orbital rim or peripheral patch layers in sheep SR.

7.3 Peripheral patch layer.

The proximal and distal peripheral patch layers are composed mainly of one type of intermediate-diameter fibre that corresponds in histochemical profile to the small G fibre of the orbital rim, but is of larger mean diameter. The largest of these intermediate

G fibres are in fact equal in diameter to the large G fibres of the central core but they are usually associated with G fibres of smaller diameter that are similar to those of the orbital rim. The mean diameter of the intermediate G fibres near to the insertion tendon is 30.0μm while at the proximal end of the distal peripheral patch layer this value falls to 18.7μm. This reduction of intermediate G fibre diameter as one passes from insertion to belly within the distal peripheral patch layer is at least in part related to the splitting of peripheral G fibres into daughter fibres which has been observed in teased preparations (see chapter 11). Near to the insertion tendon the composition of the peripheral patch layer thus almost totally consists of large-diameter G type fibres (figs.25-29). With P'ase these fibres are stained light brown with characteristic dark brown streaks which give a 'finger-print' appearance to the fibre. They are far less reactive than the C type fibres of the orbital rim. With SDH the 'pale' pattern of many small diformazan particles and streaks in a uniform network gives an overall intensity which is slightly higher than the 'white' pattern of the large A fibre, but which is still relatively low. With Alk ATPase the intermediate G fibres have minimal activity while the abundant capillary endothelia in this layer are intensely stained. The activity with Acid ATPase is intensely black. As the intermediate G fibres pass toward the belly they are associated with small C fibres similar to those of the central core and orbital rim layers and the proportion of G fibres in the layer falls to about 65%.

At the proximal peripheral patch ventral concentrations the

proportion of intermediate G fibres is even less, being about 50% within this area (figs.30-33) and the G fibres are almost uniformly small in diameter.

To summarize the histochemical fibre types in SR:-

The central core layer is composed of 7% large G (non-oxidative/non-glycolytic), 45% large A (non-oxidative/glycolytic), and 48% intermediate C and small C (oxidative/glycolytic) fibre types; the orbital rim layer in the belly of SR is composed of 66% intermediate C and small C fibre types similar to those of the central core, together with 34% small G (non-oxidative/non-glycolytic) fibres; the distal peripheral patch layer at the insertion end of SR is composed almost exclusively of intermediate G (non-oxidative/non-glycolytic) fibres. As one passes toward the belly there is an increasing percentage of small C fibres up to a maximum of 35% in this layer. The light-microscope characteristics of the various muscle fibre types in each layer of SR are summarized in Table I.

Chapter 8. Ultrastructure of fibre types in superior rectus.

8.1 The identification of fibre types in thick Epon sections.

In order to study the fine structure of the various histochemical fibre types found in the different layers of SR it was necessary to identify fibres of the same type in semi-thin (1.5μm) transverse Epon sections stained with toluidine blue. In sections taken from material post-fixed in osmium, toluidine blue stains the mitochondria intensely so that it is possible to correlate the fibre types with those fibres visible in sections stained with mitochondrial markers such as SDH, sudan black B and particularly good W & VG. In the case of SDH it is presumed that the general pattern of diformazan deposition in a particular fibre depends primarily on mitochondrial size and density, rather than on differing levels of SDH activity. The criteria used to distinguish the fibre types in Epon sections were those of: relative diameter of muscle fibres; size, density and distribution of mitochondria; fibrillar or afibrillar appearance of the myofibrils; background staining intensity of the myofibrils and sarcoplasm; and relative numbers of fibre types within a known layer of SR. Blocks of material for EM investigation were selected mainly from the extreme insertion end or the belly regions of SR. A complete semi-thin transverse section of each block enabled dorso-ventral orientation to be established and the various muscle layers to be identified.

In the central core the muscle fibres are polygonal in outline and are compactly arranged. They are divided into two broad categories that appear either high or low in overall staining

intensity, respectively (figs.36 & 39). The 'high' fibres, which possess a darker background with larger and more numerous mitochondria than the 'low' fibres, correspond to the intermediate and small C histochemical fibre types of the central core. The small C fibres are thinner and have the darkest background and the largest mitochondria. The intermediate C fibres are thicker and contain smaller mitochondria. The subsarcolemmal ring of mitochondria that characterize the intermediate C fibres in SDH preparations is less obvious in semi-thin Epon sections. Both C-type fibres in the central core exhibit a "fibrillar" appearance in transverse section with small, well-delineated myofibril bundles (fig.39). The 'low' fibres, which are of relatively large diameter and contain only few, small mitochondria, correspond in the main to large A fibres together with some large G fibres. The large G fibres may be distinguished from the large A fibres by the fact that they are usually slightly larger in diameter, occur isolated from other large G fibres, contain even fewer mitochondria, and exhibit an "afibrillar" appearance with large, poorly-delineated myofibrils (fig.39). The myofibrils of the large A fibre are well-delineated and comparable in size to those of the intermediate and small C fibres.

The orbital rim layer in the belly region is more loosely packed and has a more homogeneous appearance than the central core layer (figs.35 & 38). The homogeneous appearance derives largely from the bulk of fibres that has a dark background and numerous, large, intensely-staining mitochondria. The largest of these fibre types often shows subsarcolemmal accumulations of mitochondria

and corresponds to the intermediate C histochemical type. The smallest of these dark fibres corresponds to the small C histochemical fibre type. Between these two extremes exists a spectrum of intermediate forms. A few fibres of the orbital rim layer are extremely small in diameter and appear to correspond to the small G fibres. They exhibit low background staining with small, but still numerous, mitochondria, and are relatively pale when compared to the surrounding C fibre types. The small diameter of the muscle fibres of the orbital rim makes it difficult to ascertain the fibrillar or afibrillar pattern of the myofibrils at the light-microscope level.

The peripheral patch layer near the insertion end of SR (fig.34) is composed mainly of intermediate- to large-diameter fibres that exhibit an intermediate background staining intensity and have numerous small mitochondria. At high magnification (fig.37) these fibres, which correspond to the intermediate G fibres, have an "afibrillar" appearance with myofibrils irregularly delineated by fine streaks, and are comparable with the large G fibres of the central core.

Following identification of fibre types in transverse semi-thin sections stained with toluidine blue, ultra-thin sections were taken for viewing under the E.M., and the block face then turned through ninety degrees in order to obtain longitudinal sections of given fibre types.

8.2 Ultrastructural characteristics of fibre types.

8.21 Central core layer.

In the central core layer the striking heterogeneity of

fibre types shown in transverse semi-thin sections is also apparent in low-power electronmicrographs (figs. 40 & 41). These confirm much of what can be seen with the light microscope, and most of the additional ultrastructural information about the fibre types is derived from a study of longitudinal sections (see Table II for summary).

Of the three comparatively large-diameter fibre types in the central core (the large G, large A and intermediate C), the large G fibres appear the most homogeneous in cross-section (figs.41, 44 & 45). They are composed of large, irregularly-delineated myofibrils that are separated from each other mainly by sarcoplasm since transverse t-tubules and sarcoplasmic reticulum are only poorly-developed. The ovoid mitochondria are extremely small in transverse section and contain only 3-4 cristae. Their small size, and their sparse but even distribution throughout a cross-section of a large G fibre make the mitochondria relatively inconspicuous. The Z lines in transverse section are comparatively dense. In longitudinal section (figs.50-52) the large G fibres are again strikingly homogeneous in appearance with the large myofibrils being particularly poorly-delineated in the A band. The few, round mitochondria are located mainly in pairs on either side of the Z lines, which are fairly straight and relatively thin and possess distinct Z filaments. A prominent M line is present. The transverse tubular system and the cisternae of the sarcoplasmic reticulum form triads at the A/I band junction, but they are irregular

Table II. A summary of the ultrastructural characteristics of the various muscle fibre types in each layer of superior rectus.

Fibre types	CENTRAL CORE LAYER				ORBITAL RIM LAYER			PERIPHERAL PATCH LAYER	
	Large G	Large A	Inter. C	Small C	Small G	Inter. C	Small C	Inter. G	Small C
Myofibrils	Large, irregular	Small, well-delineated			Medium, poorly separated	Small, well-delineated		Medium, poorly separated	Small, well-delineated
Sarcoplasmic reticulum	sparse	Abundant, especially in I band			sparse	abundant		sparse	abundant
M line	present	present	present	present	absent	present	present	absent	present
Z line	narrow, straight	narrow, straight	wide, less straight	wide, wavy	wide, fairly straight	wide, less straight	wide, wavy	wide, very wavy	wide, wavy
Mitochondria:									
abundance	very sparse	sparse	moderate	abundant	moderate	moderate	abundant	moderate	abundant
location	random	peripheral	subsarco-lemmal	random	random	subsarco-lemmal	random	random	random
size	very small	small	medium	large	small	medium	large	small	large
cristae	very few	few	medium	abundant	few	medium	abundant	few	abundant
Glycogen granules	moderate	abundant	moderate	moderate	moderate	moderate	moderate	moderate	moderate
Lipid droplets	very rare	rare	moderate	common	rare	moderate	common	rare	common
'Tubular aggregates'	-	-	-	-	present	-	-	present	-
T system (triads)	infrequent, irregular	Extremely regular at A/I band junction			infrequent irregular	regular at A/I band junction		infrequent irregular	regular

and infrequent. Near to the surface of the fibres the transverse tubules may be obliquely orientated (fig.52). The sparse sarcoplasmic reticulum, which separates the large myofibrils in the I band, is flanked by moderate amounts of glycogen granules.

The large A fibres in transverse section (figs.42 & 43) have small, regular myofibrils that are particularly well-delineated at the level of the A/I band junction by extensive transversely-orientated tubules, and in the I band by single layers of tubules of sarcoplasmic reticulum. The ovoid mitochondria are slightly larger than those of the large G fibres, but are still relatively small with few cristae. They are relatively infrequent and are scattered throughout a cross-section of the fibre, although they tend to form subsarcolemmal accumulations. In longitudinal section (figs.53-55) the mitochondria are arranged mainly in pairs on either side of the Z line, or in longitudinal rows between the myofibrils, or beneath the sarcolemma. The Z line is thin and straight with distinct Z filaments, and a prominent M line is present. The transverse tubular system is well-developed and triads occur regularly at the A/I band junction. Large amounts of glycogen granules are associated with the abundant sarcoplasmic reticulum in the I band. Lipid droplets are rare.

The intermediate C fibres in transverse section (figs.48 & 49) also have small myofibrils that are well-delineated at the A/I band junction by transverse tubules and by abundant sarcoplasmic reticulum in the I band. The mitochondria are larger in cross-section than those of the large A fibre and are more numerous.

Their distribution pattern is similar to that of the large A fibre although the subsarcolemmal accumulations are more striking. In longitudinal section (figs.56 & 58) the mitochondria appear elongate and have characteristic tightly-packed cristae. Chains of mitochondria, each of which may extend through up to three sarcomeres, lie between the myofibrils and may be associated with lipid droplets. The Z lines are appreciably thicker than those of either the large G or the large A fibres, although they also show distinct Z filaments. The Z lines of the intermediate C fibres appear slightly wavy. An M line is present, although it is not as prominent as in the large G or large A fibre types. The well-developed sarcoplasmic reticulum forms remarkably regular triadic junctions with the transverse tubules at the A/I band junction, and is associated with moderately high amounts of glycogen granules.

The ultrastructure of the small C fibres of the central core is comparable with that of the intermediate C fibres except that their diameter is much smaller. In transverse section (figs.46 & 47) the small C fibres show small, well-delineated myofibrils with abundant sarcoplasmic reticulum and extensive transverse tubules at the A/I band junction. The mitochondria are large with tightly-packed cristae and, in contrast to the intermediate C fibre, are distributed evenly throughout a transverse section. In longitudinal section (figs.56 & 57) the elongate mitochondria are arranged in rows between the myofibrils and are often associated with lipid droplets. An M line is present; the Z lines are relatively wide and wavy although Z filaments are shown; there is abundant glycogen.

8.22 Orbital rim layer.

Low-power electron micrographs of the orbital rim layer in the belly of SR (fig.59) show an array of fibre types that are mostly rich in mitochondria, and which, in respect of their mitochondrial patterns and diameters, correspond to the intermediate C and small C fibre types of the central core. A few very small-diameter G fibres are also present that contain very small mitochondria and have a uniform appearance in transverse section. The intermediate C and small C fibres of the orbital rim both exhibit small myofibrils that are well-delineated in the A/I band junction by extensive transverse tubules, and in the I band they are surrounded by a single layer of tubules of sarcoplasmic reticulum (figs.62-65). The myofibrils appear to be less delineated by sarcoplasmic reticulum in the A band compared with the C fibre types of the central core layer (compare figs.46-49). In longitudinal section (figs.66 & 67) the ultrastructure of the C fibre types in the orbital rim layer is similar to the respective C fibre types in the central core layer. The elongated mitochondria have tightly-packed cristae and are arranged in intermyofibrillary chains, often in association with lipid droplets; the thick Z lines may be slightly wavy although they have distinct Z filaments; faint M lines are present within a pseudo-H zone in the centre of the A band; triads occur regularly at the A/I band junction; and there are moderate amounts of sarcoplasmic reticulum in the I band

with associated glycogen granules. The C fibre types of the orbital rim layer in the belly of SR tend to form a spectrum rather than distinct intermediate C and small C types as in the central core.

The small-diameter G fibres from the orbital rim layer in the belly of SR (figs.59 & 60) have mitochondria that are much smaller in diameter than those of the neighbouring C fibre types and which are relatively numerous. The mitochondria are distributed evenly throughout a transverse section without any subsarcolemmal accumulations in a similar pattern to that of the large G fibres of the central core, although they are slightly larger and more numerous. The uniform appearance of the small G fibres in transverse section derives partly from the small, relatively inconspicuous mitochondria, and partly from the poor delineation of the myofibrils, which are only partially delimited by sarcoplasmic reticulum in the I band and are not delimited at all in the A band (fig.61). Where the myofibrils are visible they are smaller than those of the large G fibre of the central core. The transverse tubular system is not abundant and does not markedly delineate the myofibrils as in the other fibre types of the orbital rim, although triads are occasionally visible in transverse sections. In longitudinal section (figs.68 & 69) the ovoid mitochondria either form pairs on either side of the Z band or may extend across a sarcomere to form short chains with neighbouring mitochondria. There are occasional lipid droplets associated with these short chains. The Z band is relatively wide, fairly straight and contains

distinct Z filaments. An M line is absent from the pseudo-H zone. The poorly-developed sarcoplasmic reticulum is restricted to the I band and is associated with moderate amounts of glycogen granules. Triads, although relatively few in number and irregular in occurence, are more numerous than in the large G fibre of the central core. They are located at the A/I band junction.

At a level between the belly and insertion of SR, the small G fibres of the orbital rim are generally larger in diameter (figs.70-72) although their ultrastructural characteristics remain identical to those of the small G fibres in the belly. With increased diameter the small G fibres appear even more homogeneous.

Some of the small G fibres of the orbital rim have been observed to contain aggregations of tubule-like structures that lie immediately beneath the sarcolemma (figs.73-75). Such structures appear in transverse section as single-membrane tubules that contain an inner tubule with two membranes and which is comparatively electron dense.

At the insertion end of SR the orbital rim layer contains, in addition to the spectrum of C fibre types and small G fibres already described, large A fibres that correspond in ultrastructure to the large A fibres of the central core layer.

8.23 Peripheral patch layer.

The peripheral patch layers are easily distinguished in

low-power electron micrographs (fig.76) since they are composed almost entirely of medium- to large-diameter G type fibres that are characteristically well-separated from each other by extensive connective tissue. The numerous mitochondria are small in diameter with only a few cristae, and are scattered evenly throughout a cross-section. The myofibrils are only poorly delineated in the I band and not delineated at all in the A band (figs.77-78). The intermediate G fibres of the peripheral patch thus appear relatively homogeneous in transverse section.

In longitudinal section (figs.79-80) the fine structure of the peripheral patch G fibres resembles that of the small G fibres of the orbital rim. The Z band is thus wide and contains distinct Z filaments; mitochondria are small and are arranged either in pairs on either side of the Z line or they may extend across a sarcomere; an M line is absent from the middle of the A band; tubules of sarcoplasmic reticulum with associated glycogen granules are poorly-developed and only present in the I band; triads do occur at the A/I band junction but are infrequent.

In these larger-diameter G fibres of the periphery the Z band often appears more wavy than in the small G fibres in the belly region of the orbital rim.

A summary of the ultrastructural characteristics of the various fibre types in each layer of SR is given in Table II.

Chapter 9. Types of motor nerve ending and their distribution in superior rectus.

9.1 Cholinesterase preparations.

Cholinesterase activity in SR is localised not only on extrafusal muscle fibres as motor endings, but also at muscle-tendon and muscle-muscle junctions, which are described later (9.15).

9.11 Plate and grape endings.

A low-power examination of a superior rectus muscle that has been stained *in toto* to demonstrate cholinesterase activity shows two major types of motor ending on the extrafusal muscle fibres (fig.81). The first type consists of intensely-staining ovoid structures 15-35μm in diameter that resemble the typical motor end-plates of other striated muscle. Such plate ('en plaque') endings in SR are scattered in clusters throughout the length of the muscle. Each cluster of plate endings occurs at approximately the same level on adjacent muscle fibres, but there is no discrete, central terminal innervation band as in the extraocular muscles of the majority of other mammalian species (1,2). The arrangement of plate and grape endings in sheep SR corresponds to that shown in the extraocular muscles of the cynomolgous monkey (Hess, 1962), and the scattered pattern of innervation suggests that some muscle fibres do not extend the full length of the muscle. The plate endings

may be distinguished under the dissecting microscope after only 5-10 minutes incubation. In teased, or squashed, preparations of material given such short incubation the complex synaptic guttering underneath the motor end-plates can be clearly seen (figs.87 & 89). Increased incubation time results in the loss of such fine detail because of increased deposition and diffusion of reaction product, and the motor end-plates appear as 'discs' of cholinesterase-positive material. The plate endings possess both high acetyl-cholinesterase (AChE) and butyrylcholinesterase or non-specific esterase (BChE) activity.

The second type of ending is more diffuse and is more variable in form, being composed of very small cholinesterase-positive droplets. Such grape ('en grappe') endings appear at low magnification as specks of lower intensity scattered between the clusters of highly-reactive motor end-plates (fig.81). The grape endings possess weaker AChE and BChE activity than the plate endings and need at least 20 minutes incubation before they become visible. Consequently, in material incubated primarily to demonstrate the diffuse grape endings, the compact plate endings are usually overstained and the synaptic guttering obscured.

9.12 Distribution of endings throughout the various layers.

The separation of individual muscle fibres from small muscle-fibre bundles teased specifically from the three layers of SR shows that each layer contains both plate-innervated and grape-

innervated fibres. The plate innervation is single, with one motor end-plate being supplied to any one muscle fibre, and the grape innervation is multiterminal with several zones of grape endings being scattered along a particular muscle fibre. The bundles of muscle fibres from the central core are composed mainly of plate-innervated fibres that are mostly of intermediate to large diameter, but which also include small-diameter fibre types. There are also about 7% of grape-innervated fibres that are among the largest-diameter fibres in the central core. The orbital rim layer also contains mainly plate-innervated fibres of small to intermediate diameter, with about a third of small-diameter grape-innervated fibres that possess a more profuse innervation than the grape-innervated fibres of the central core. The peripheral patch layer at the insertion end of SR is almost entirely composed of grape-innervated fibres of intermediate to high diameter. This concentration of multiterminally-innervated fibres is particularly striking since the innervation, like that of the grape-innervated fibres of the orbital rim, is very profuse.

9.13 Two patterns of grape innervation.

The proportions and relative diameters of the grape-innervated fibres in each of the SR layers corresponds to those of the histochemical G-type fibres found in the central core, orbital rim and peripheral patch layers. The pattern of grape innervation of the central core G fibres is different from that of the orbital

rim and peripheral patch G fibres, as has already been outlined (Barker & Harker, 1972). A sample of 38 peripheral patch G fibres were teased from the dorsal layer of SR at the insertion end and isolated for as great a distance as possible (see fig.83). The extensive connective tissue of this layer makes this a difficult task, although portions of muscle fibres were obtained containing up to five 'innervation zones'. These 'zones' are composed of irregular chains of cholinesterase-positive droplets that provide an almost continuous innervation over a given length of muscle fibre (fig.85) and which are irregularly spaced apart. The cholinesterase-positive droplets are of variable size and form. They range from small-diameter ovoid structures to larger irregularly-shaped forms, which often spiral extensively around the muscle fibre. The 38 muscle fibres had a mean diameter of 33.0μm; the 94 'innervation zones' extended on average for about 200μm along the fibre and contained between 5 and 38 droplets (mean = 16.5). The 'innervation zones' were spaced apart by an average distance of about 610μm.

The central core G fibres (fig.84) show small compact 'innervation zones' composed of clusters of small cholinesterase-positive droplets, round or oval in shape, which are usually quite discrete. There is no complex synaptic guttering comparable to that associated with the compact motor end-plate (figs.86 & 88). The grape endings of the central core G fibres do not stain as intensely as the grape endings found in the peripheral fasicles of SR. In a sample of 26 central core G fibres the 105 'innervation

zones' extended on average for about 60μm along the muscle fibre, contained about 7 droplets, and were spaced apart by an average distance of 860μm. The mean-diameter of the muscle fibres was 39.0μm which was the largest mean diameter of the cholinesterase-stained material.

In a sample of 10 isolated orbital rim G fibres taken from the belly of SR, the mean diameter of the muscle fibres was 22.0μm, the average length of the 'innervation zone' was 100μm, and these zones were spaced apart by an average distance of 400μm. The form of the grape endings resembled that of the peripheral patch G fibres, but the average number of cholinesterase-positive droplets in the innervation zones was lower (8-9). Some of these small-diameter orbital rim G fibres are probably derived from larger-diameter peripheral patch G fibres that subdivide as they pass from the insertion to the belly of the muscle (chapter 11). The peripheral patch and orbital rim G fibres may thus form a peripheral grape-innervated component quite distinct from that located in the central core. This peripheral component appears to have the most profuse grape innervation concentrated in the peripheral patch layers at the extreme ends of the muscle. Central core G fibres have not been observed to subdivide; their 'innervation zones' are shorter, contain fewer and smaller cholinesterase-positive droplets, and are spaced farther apart than in the peripheral G fibres.

9.14 Pharmacology of endings.

The plate endings, and both types of grape ending, all show AChE and BChE activity. There appeared to be no difference at all in the case of plate endings between the activity exhibited towards acetylthiocholine and that towards butyrylthiocholine. In the case of grape endings gross activity is lower and activity towards acetylthiocholine tends to be slightly higher than that towards butyrylthiocholine.

These findings are similar to those reported for the extraocular muscles of the cynomolgus monkey (Hess, 1962), cat (Hess & Pilar, 1963), rhesus monkey (Silver, 1963; Zenker & Anzenbacher, 1964), guinea pig, goat and rabbit (Silver, 1963), man (Dietert,1965), and rat (Teräväinen, 1968a).

9.15 Additional cholinesterase-positive structures.

At the origin and insertion ends of SR the muscle fibres that form the musculo-tendinous junctions display a 'palisade' of cholinesterase-positive material around their ends (fig.90). This palisade resembles the 'cholinesterase cuffs' described by Couteaux (1953) in skeletal muscle. Similar structures have been observed at the musculo-tendinous junctions of rat eye muscles (Mayr, 1971) and human eye muscles (Kupfer, 1960; Cheng, 1963).

Within the peripheral patch and orbital rim layers of SR there are also a variety of cholinesterase-positive muscle fibre

interconnections, both with and without the interposition of tendinous material (figs.91-92). These interconnections take the form of end-to-end junctions between either two undivided fibres that do not extend the full length of SR, or, more commonly, between an undivided fibre and a short branch derived from a peripheral G-type fibre. These fibres, as they pass towards the belly of the muscle, reduce in diameter by either forming a "Buttress-like" attachment point (fig.92) or by subdividing into two daughter fibres, one of which may form a short branch (fig.91). Cholinesterase activity is localised both around the end of the attached fibre and around the point of attachment and resembles the 'palisade' of cholinesterase-positive material at the musculo-tendinous junction described above. The muscle fibre/ muscle fibre junctions may have little tendinous material interposed between the ends of the two muscle fibres (figs.91 & 95), or they may be separated by 100μm or more of tendon (figs.92 & 96). The junctions shown in cholinesterase preparations are compared with similar junctions seen in teased, silver preparations (figs.95 & 96). A tendinous attachment of a small-diameter extrafusal muscle fibre to a larger diameter one without any buttressing or end-to-end junctions is shown in fig.94. This preparation is of teased, silver-impregnated material.

It is evident that in sheep SR a complex, three-dimensional network is built up in the peripheral layers by the interconnection of muscle fibres. A comparable network of muscle fibres that are interconnected by short-tendon attachments and tendon-free junctions

has been described in the iris muscle of the chicken (Zenker & Krammer, 1967), an inner eye muscle. The branched muscle fibres of the avian iris are also grape-innervated, as are the peripheral G fibres in sheep SR, but have an internal structure typical of fast fibres (Hess, 1966).

End-to-end junctions of (unbranched?) muscle fibres with palisades of cholinesterase-positive material interposed between have been reported in the extraocular muscles of the cat (Floyd, 1970), the rat (Mayr, 1971) and the serin finch (Mayr, Zenker & Gruber, 1967).

9.2 Silver preparations.

An examination of teased, silver preparations taken from the various layers of SR shows that the main nerve trunk enters the fascia posteriorly along the lateral border (fig.3) and divides as it passes at right angles to the length of the muscle to give smaller nerve bundles. These course parallel to the muscle fibres toward either the origin end of the muscle or, mainly, toward the insertion on the eyeball.

9.21 Plate endings.

The extrafusal motor end-plates that are scattered in groups throughout the length of SR are derived from medium- to large-diameter myelinated axons that leave the small nerve bundles, pass at 90^{o} over the muscle fibres and divide to give a spray of up to twenty end-plates (fig.98). Immediately adjacent to the end-plate

the myelinated axons in the silver preparations have a total diameter of 3.0-5.9µm (mean = 4.28). Measurements were made immediately proximal to the last division of the nerve prior to the end-plate. The end-plates range in complexity from the simplest, or T1 type (Tuffery, 1971), which is formed from an unbranched terminal axon (figs.98-100), to more complex end-plates that are formed from two, three or more myelinated branches of the terminal axon (giving T2, T3 etc. forms) such as is shown in figs.101-104. Such complex end-plates occur only rarely in other normal skeletal muscle as a consequence of growth and elaboration (Tuffery, 1971). Also unlike the end-plates of other skeletal muscles it is common, even in the simpler forms, to observe myelinated nerves within the region of the end-plate (fig.100). In the terminal course of such nerves the internodal distances are very small and unmyelinated terminal branches leave the main nerve at the nodes of Ranvier to contribute to the end-plate region. Such naked axon terminals appear as arboreal branchings often with terminal axoplasmic swellings or loops (fig.99). The complexity of an end-plate configuration in terms of number of contributing myelinated branches of the terminal axon does not appear to be related to muscle-fibre diameter. Thus the thin, plate-innervated muscle fibres of the orbital rim layer may often possess endings with a T1, T2 or T3 form (fig.104).

9.22 Grape endings.

In addition to the small bundles of large-diameter nerve

fibres that supply sprays of end-plates to restricted zones of SR there are also present throughout the muscle axons of small diameter that can occur either as single isolated axons or as small nerve bundles which terminate in multiterminal 'grape' endings of variable appearance. These axons run in a direction approximately parallel to the muscle fibres, though their course is often a meandering one, and they branch frequently to produce a complex network of nerves in some zones of the muscle. The density of these small-diameter nerves and their terminations is particularly striking in the peripheral patch layer at the insertion end of SR; is lower in the peripheral fascicles of the orbital rim layer; and is lowest in the fascicles of the central core.

9.221 Peripheral grape endings.

The thinly-myelinated axons that form the complex network in the peripheral patch layer at the insertion end of SR are, on average, about 2.5μm in diameter. This measurement is prior to their final branching to give non-myelinated terminal fibrils. These fibrils terminate in grape endings that are generally the most complex in the muscle. In the most compact of these terminations the terminal fibrils divide repeatedly to end in small knobs or axoplasmic swellings that produce the characteristic 'bunch of grapes' appearance (figs.105 & 108). Other grape endings in the peripheral patch layer appear more linear in form with the non-myelinated terminal fibril passing along individual muscle fibres, sometimes for considerable

distances (fig.106). At regular intervals extremely fine branches are given off and end in small knobs, and the terminal portion of the main fibril may also contain axoplasmic swellings. Since both of these types of grape ending have occasionally been seen to be derived from a common axon (fig.107) it is assumed that they represent extremes of a spectrum with all possible intermediate forms. The similarity of distribution of the axoplasmic swellings and the cholinesterase-positive structures located on the peripheral patch G type fibres (see e.g. fig.83) suggests that the swellings constitute the major points of synaptic contact with the underlying muscle fibre.

Within the peripheral patch layer the linear form of grape ending becomes the more common type as one passes toward the belly of the muscle and the peripheral patch G fibres are reduced in diameter. The relatively extensive innervation zones of this part of the muscle may occasionally be formed by several non-myelinated terminal fibrils that are derived from the same parent axon and each of which terminates in a fairly simple ending (fig.109).

Within the orbital rim layer in the belly of SR the multi-terminally-innervated muscle fibres are generally of small-diameter and their grape endings are linear in form (figs.110-111).

9.222 Central grape endings.

In the central core layer axons of small diameter are

relatively infrequent compared to the other layers of SR. Prior to their final branching these nerves are about 2.5μm in diameter and appear to be thinly-myelinated. They course for considerable distances through the central core layer and branch to supply infrequently-occuring muscle fibres of large-diameter that are presumed to be central core large G fibres. In silver preparations these muscle fibres often appear paler than the surrounding plate-innervated ones. The thinly-or non-myelinated terminal fibrils form fairly compact innervation zones that are more widely spaced apart than those of the peripheral grape-innervated muscle fibres. The main fibrils often spiral around the muscle fibre before terminating in fairly coarse knobs or axoplasmic swellings (figs.112-114 & 118-120). Extremely fine terminal branches are also sometimes discernable that terminate in very fine knobs or loops (figs.115-116). It is possible that these extremely fine terminations are only occasionally impregnated with silver. This would explain the apparent sparsity of points of synaptic contact in the silver preparations of central core G fibres when compared with cholinesterase preparations (figs.86 & 88). The most compact of the central core grape terminations may closely resemble the form of the classical motor end-plate of skeletal muscle in that the terminal fibrils branch only once or twice and end in thickenings or swellings among several nuclei. In one of the 'plate-like' endings illustrated (fig.117) a fine branch from the parent axon passes further along the muscle fibre and ends in several knobs and loops. Such configurations may be distinguished from true end-plates by the fact that their parent

axons are never as thick, and also because they occur on muscle fibres that are multiterminally innervated. The variety of form of the central 'compact' grape endings is illustrated in figs.112-120. Terminations can range from extremely simple bifurcated fibrils with only three or four visible points of synaptic contact (fig.115) to more extensive endings in which the terminal fibrils take a longer course and are closely applied to the underlying muscle fibre.

Chapter 10. Ultrastructure of the extrafusal myoneural junctions in superior rectus.

A total of 58 extrafusal myoneural junctions were examined in either longitudinal or transverse section under the electron microscope. The number of endings that were examined for the different muscle-fibre types in each of the three layers of SR is given in Table III. Two major types of ultrastructure are shown corresponding to the plate and grape types of innervation. The singly-innervated muscle fibres show neuromuscular junctions comparable in ultrastructure to the typical motor end-plates of mammalian skeletal muscle. The axon terminals are located in synaptic grooves with numerous junctional folds, and in SR they are applied only to those muscle fibre types that exhibit a 'twitch-type' morphology (Hess, 1970; 1967), that is, to large A, large C and small C fibre types. The second type of motor junction is applied to the multiterminally-innervated large G fibre of the central core, and the intermediate G and small G fibres of the periphery. The axon terminals of these endings are located either in shallow grooves or on the surface of the muscle fibre with only rudimentary or irregular junctional folds. The ultrastructural characteristics of the myoneural junctions applied to the various fibre types is summarized in Table III.

Table III. Ultrastructural characteristics of the myoneural junctions applied to the various fibre types in superior rectus.

Muscle Fibre Types	CENTRAL CORE LAYER				ORBITAL RIM LAYER			PERIPHERAL PATCH	
	Large G	Large A	Inter. C	Small C	Small G	Inter. C	Small C	Inter. G.	Small C
Numbers of myoneural junctions observed	5	4	8	4	4	5	7	18	3
Axon terminal:									
size	small	large	large	large	small	large	large	small	large
grouping	compact	compact	compact	compact	isolated	compact	compact	isolated	compact
synaptic vesicles (sv)	sv	sv	sv	sv	sv	sv	sv	sv	sv
dense-core vesicles (dc)	dc	dc	dc	dc	dc	dc	dc	dc	dc
Guttering on muscle surface	absent	present	present	present	absent or shallow	present	present	absent or shallow	present
Sole-plate	thin	thick	thick	medium	absent or thin	thick	medium	absent or thin	medium
Myoneural junction	mainly smooth	regularly folded	regularly folded	mod. folded	irreg. folded	regularly folded	mod. folded	irreg. folded	mod. folded
Form of junctional folds	wide & irregular	thin, straight	thin, straight	expanded at base	curved or vacuolar	thin, straight	expanded, irregular	curved or vacuolar	expanded & irregular

10.1. Plate endings.

The axon terminals of the motor end-plates in SR are derived from myelinated large-diameter motor axons, that have prominent mitochondria, neurofilaments and neurotubules, and which are often visible in a transverse section through the end-plate region (see, for example fig.124). The terminals are characteristically compact in their arrangement. In transverse section up to half the perimeter of a particular muscle fibre may be covered with axon terminals (see figs.124 & 128) which lie in synaptic gutters above a sole plate with regular and frequent post-synaptic folds.

The neuromuscular junctions of the large A fibres of the central core (figs.121-123) are usually located on a pronounced Doyère's emminence with a relatively thick sole plate and associated sole plate nuclei (fig.123). The axon terminals are large and frequently elongate and they extend over relatively large areas of post-synaptic membrane. The junctional folds are remarkable for their great regularity and abundance. They are closely spaced and generally uniformly narrow with only occasional expansions near their base (fig.122). They also tend to be single folds, although branching does occur.

The end-plates of the intermediate C muscle-fibre types of the central core (figs.124-125) and orbital rim (figs.126-127) layers are also applied to pronounced sole plates, although these are not as thick as in the large A fibres. In other respects the fine structure of the axon terminals and junctional folds resembles

that of the large A fibre end-plate. The junctional folds are thus mainly narrow and only occasionally expanded near their base (fig.127). The frequency of folding appears to be slightly less than in the large A fibre type.

The plate endings of the small C fibres of the central core, orbital rim and peripheral patch layers overlie the thinnest sole plates and possess the most irregular junctional folding of all the plate endings (figs.128-130). The folds are frequently expanded at their base and thus appear more 'vacuolar' than those of the large A and large C plate endings.

In skeletal muscle fibres similar differences in the fine structure of motor end-plates have been demonstrated in rat (Ogata, Hondo & Seito, 1967; Padykula & Gauthier, 1970), human (Murata & Ogata, 1969) and mouse (Duchen, 1971) material.

10.2 Grape endings.

The neuromuscular junctions of the intermediate G and small G fibre types of the peripheral patch and orbital rim are formed by axon terminals that are much smaller than those of the end-plate junctions (compare figs.133 & 122). Even when several of these axons are present in a given transverse section (figs.131 & 143) they do not occupy a great proportion of the periphery, and it is equally as common to find single, isolated terminals (fig.132). Small-diameter, thinly myelinated axons are seen in close proximity to the endings (fig.142) with non-myelinated axons more common nearer to the surface of the muscle fibre. The axon terminals are usually applied to the surface of the muscle fibre, but may occasionally be

located in shallow synaptic gutters (fig.141). There is usually little or no sole-plate material beneath the terminals and the synaptic gutter is mainly smooth with only infrequent and irregular junctional folding. Any deep invagination of the sarcoplasmic membrane is 'flask-shaped' with the folds expanded at their base and these folds do not extend straight into the sarcoplasm, but are usually curved. Such folds can appear as 'vacuoles' lined by basement membrane that lie beneath the post-synaptic membrane (fig.133). Serial transverse sectioning confirms that these endings on peripheral G type muscle fibres are multiterminal in nature.

The nerve endings that supply the large G fibres of the central core are similar in most respects to those of the peripheral G muscle fibres except that in longitudinal section the terminal axons are more compactly arranged (fig.134). The endings are applied to the surface of the muscle fibre and overlie a thin sole plate. Junctional folds are rare and when present are irregular in form. Such folds are obliquely orientated, bulbous and occasionally branched (fig.137). The axonal protrusions seen in the grape terminals of rat (Teräväinen, 1969) and cat (Cheng & Breinin, 1965) are not observed in sheep.

Apart from the differences in the amount and form of junctional folds and the thickness of the sole plate in the various endings, the arrangement of organelles and basic fine structure of the junctions is similar for all the ending types. The axon terminals thus contain: numerous clear 'synaptic' vesicles of about 400-500Å in diameter that are present throughout the axoplasm, but are

especially numerous near the junctional region itself; small ovoid mitochondria that tend to occur in clusters; and neurotubules that are found away from the junctional region. In addition, dense-core vesicles of 700-800Å in diameter, with cores of about 500-550Å diameter, have occasionally been observed in the axon terminals supplying large A (fig.122) large C (fig.127) and small C (fig.129) "twitch" fibre types, as well as in the terminals of the peripheral G (fig.133) and central large G (figs.135-136) "slow" muscle fibre types. The axoplasmic membrane of all the axon terminals is separated from the sarcoplasmic membrane by a gap of 625-1,000Å with basement membrane material 150-200Å thick interspersed between. This basement membrane extends into the junctional folds, when present, and separates the two sarcoplasmic membranes that face each other. In the terminals of the large A muscle fibre the basement membrane remains single in most of junctional folds since they are narrow. In the expanded folds in the terminals of the C and G muscle-fibre types the basement membrane separates to give a 'vacuole'. Where a sole plate is present beneath a nerve ending, ribosomes, sole-plate nuclei, and small mitochondria accumulate.

Chapter 11. Longitudinal division of peripheral patch G fibres in superior rectus.

11.1 Teased preparations.

The isolation of individual peripheral patch G fibres teased from the insertion end of SR, in either silver or cholinesterase preparations, shows that as these fibres pass towards the belly of the muscle they undergo a reduction in diameter, usually with an associated increase in muscle-fibre number, by dividing into two daughter fibres (figs.83, 93 & 97). These are each roughly half the diameter of the parent fibre, and they may each continue for an appreciable distance (i.e. the limits of teasing without breaking); or else one of the branches is very short and serves for the tendinous attachment of another fibre (figs.91 & 95). In the latter case the attached fibre is most commonly a small C fibre. The longitudinal division of the peripheral patch G fibres and the associated tendinous attachment of small C fibres produces a complex three-dimensional network in the peripheral patch layer at the insertion end of SR.

In teased, silver preparations (fig.97) the continuity of the parent fibre and daughter fibres may be ascertained by the continuity of both the cross striations and staining intensity of the muscle fibre. The cross striations can usually be traced back to some common source and the G fibres of the peripheral patch layer are generally paler staining than the small C fibres with which they are associated. In teased, cholinesterase preparations (fig.93)

the distinction between a 'true' longitudinal division of a muscle fibre and a tendinous attachment is made easier since the musculo-tendinous attachment points are cholinesterase-positive and give characteristic 'palisades' (figs.91-92). By the use of phase microscopy the continuity of the cross striations in a dividing muscle fibre may be seen (fig.93).

11.2 Electron microscopy.

An electron-microscope study of the intermediate G fibres of the peripheral patch layer was made as they passed toward the belly of SR. Semi-thin (1.5µm thick) transverse sections stained with toluidine blue were alternated with ultra-thin sections for viewing at high power. Several of the intermediate G fibres were observed to divide into daughter fibres (figs.138-143). These fibres first constricted into a 'dumb-bell' shape before 'pinching' into two separate fibres each of which formed a continuous basement membrane around their periphery. The intermediate G fibre illustrated is innervated by a simple grape motor terminal, which lies in a shallow gutter on the sarcoplasmic membrane and has only irregular and sparse junctional folds (fig.141).

The present study thus confirms Tergast's (1873) observations of branched muscle fibres in sheep extraocular muscle. Similar observations have been made in the eye muscles of cat and rhesus monkey (Sherrington, 1894), horse (Dogiel, 1906) and rabbit (Hines, 1931). In rabbit the direction of branching is

toward the origin or insertion end of the muscle, which is the opposite direction to that seen in sheep.

Whereas in sheep SR branching has been observed only in the peripheral G fibres, branched fibres with a fast-type morphology and central location have been described in the extraocular muscles of the chicken (Hess, unpublished observations) and gudgeon (Kordylewski, 1974).

In hindlimb skeletal muscles longitudinal division of muscle fibres is usually attributed to a pathological condition (see e.g. Bell & Conen, 1968) or the response of a muscle to prolonged heavy training (see e.g. Hall-Craggs, 1970; Reitsma, 1970). However, branching of muscle fibres does occur in normal human spinal muscle (Susheela, 1964), in the intrafusal muscle fibres of cat spindles (Barker & Gidumal, 1960; 1961; Boyd, 1962), and also in normal rat soleus muscle (Edgerton, 1970).

Chapter 12. Levator palpebrae.

12.1 Gross organization.

At any point along the length of LP a transverse section, stained with W & VG, shows essentially the same gross organization. The muscle appears uniform and is not divided into the two major layers present in the rectus and oblique extraocular muscles (i.e. the central core of large-diameter muscle fibres and the orbital rim of small-diameter muscle fibres.). This impression is largely confirmed by the use of histochemical stains. In a comparable study to that carried out on SR, frozen transverse sections were sampled at 1mm intervals from a whole LP and stained to demonstrate P'ase activity. All transverse sections at low magnification (fig.144) show LP to be composed of mixed histochemical fibre types. There are no distinct layers comparable to those occuring in SR, and no proximal and distal peripheral patch layers as occur at the origin and insertion ends of SR. There are, however, minor differences in the proportions of muscle-fibre types between the central and peripheral fascicles (see Table IV). The central fascicles of LP are composed of about 55% of large-diameter fibres that are the most intensely staining for P'ase; about 25% of medium-diameter fibres that are intermediate to high for P'ase; and about 20% of small-diameter fibres that are low in intensity for P'ase. These small fibres appear to be more common within the peripheral fascicles, and this is more clearly demonstrated in transverse sections that have been processed to demonstrate actomyosin ATPase after acid pre-incubation (Acid ATPase). In such preparations

Table IV. Light-microscope and ultrastructural characteristics of the fibre types present in levator palpebrae.

Fibre Types	Large A	Inter. C	Small B
Relative diameter	Large	Medium	Small
P'ase activity	High	High	Low
SDH activity	Low	High	High
Alk ATPase activity	High	High	Low
Acid ATPase activity	Low	Low	High
Fibrillar pattern	'Fibrillenstruktur'		
Innervation	Single plate ('en plaque') innervation		
% peripheral fibres	40	15	45
% central fibres	55	25	20
% combined total	51	26	23
Myofibrils	Small, well-delineated, especially in I band		
Sarcoplasmic reticulum	abundant		
T system (triads)	regular at A/I band junction		
M line	present	present	present
Z line	thin, straight	intermediate, less straight	thick, wavy
Mitochondria:			
abundance	infrequent	moderate	abundant
location	throughout TS	subsarcolemmal	throughout TS
size	small	medium	medium
Glycogen	abundant		
Myoneural junctions			
Number observed	5	7	5
Size of axon terminal	large,elongate	large	small
Depression of muscle	gutter	gutter	gutter
Sole plate	substantial	moderate	thin
junctional folds	regular, thin straight folds	less regular folds, some bulbous	irregular folds bulbous

(fig.145) the small fibres that are low with P'ase are now stained an intense black and can clearly be distinguished from the remaining pale-staining muscle fibres. The small-diameter fibres form small groups and columns that extend between the other larger-diameter fibres of the periphery. The spindles are randomly located within a given transverse section and are not distributed around the periphery as in SR. Distinct layers are also absent from the LP muscles of man, ape, dog, cat and rabbit (Kato, 1938), baboon (Durston, 1974) and various birds (Maier et al.,1972).

12.2 Histochemical fibre types.

The extrafusal muscle fibres composing LP can be divided into three major types on the basis of diameter and histochemical profile (figs.146-149).

The largest diameter fibre type in LP possesses a type A histochemical profile. These large A fibres predominate in the centre of the muscle where they are commonly located around the periphery of fascicles. Their activity with P'ase (fig.146) is high with a grey-blue colouration of the sarcoplasmic network around the myofibrils. With SDH (fig.147) they display a 'white' pattern of fine formazan particles that are mainly localised around the periphery of the muscle fibre leaving the centre relatively clear. The activity after staining with Alk ATPase (fig.148) is high with a black reaction product, whereas with Acid ATPase (fig.149) this staining pattern is reversed and the fibres show minimal activity. The large A fibres thus possess a glycolytic/non-oxidative type of

histochemical profile. In the central fascicles they constitute about 56% of the total number of fibres present (a total of 386 muscle fibres were counted), whereas in the peripheral fascicles the figure falls to about 39% (a total of 369 muscle fibres were counted).

The second largest fibre type in LP shows a type C histochemical profile and an intermediate diameter, and in these respects may be compared with the intermediate C fibres of SR. These intermediate C fibres in LP show high activity with P'ase which in colouration is dark-brown and may generally be distinguished from the high activity shown by the large A fibres. With SDH the pattern of staining is 'intermediate' with subsarcolemmal concentrations of formazan particles in addition to some central particles. In LP the SDH reaction is not as intense as in SR, and a comparison of the intermediate C fibres of LP and SR also shows that the formazan particles are not as large in LP. The activity of the intermediate C fibres in LP with Alk ATPase is almost as high as that of the large A fibres. With Acid ATPase the activity is low, but not minimal. The intermediate C fibres constitute about 26% of the fibres in the central fascicles, and about 16% of the fibres around the periphery of the muscle.

The third extrafusal fibre type in LP is the smallest in diameter and possesses a type B histochemical profile not present in SR. With P'ase these small B fibres show a low activity and the reaction product is light-brown or yellow in colouration. With SDH they show a high activity with formazan particles scattered

evenly throughout the transverse section of the fibre with no appreciable rim of high activity as in the intermediate C fibre. The SDH pattern resembles that of the small C fibre of SR except that the particles are smaller and the reaction is less intense overall. The small B fibres show minimal activity with Alk ATPase, whereas with Acid ATPase this level of activity is reversed to high with an intense black reaction product. The small B fibres constitute about 20% of the fibres in the central fascicles and about 45% of the fibres from the periphery.

From the combined count of 705 muscle fibres from central and peripheral fascicles in LP about 51% are large A fibres; 23% are intermediate C fibres; and about 26% are small B fibres. Thus, in terms of cross-sectional area the bulk of LP is composed of glycolytic/non-oxidative large A fibres together with glycolytic/oxidative intermediate C fibres. The non-glycolytic/oxidative small B fibres form a significant proportion of the bulk only around the periphery, where they are easily distinguishable in terms of their Acid ATPase reaction (see Table IV for summary). The large A, intermediate C and small B fibre types in sheep LP correspond in diameter and histochemical profile to the 'granular','coarse' and 'fine' fibre types, respectively, in the LP muscles of man and baboon (Durston, 1974).

In addition to these extrafusal muscle fibre types there are also present in transverse sections of LP muscle fibres of extremely small diameter that show low activity with P'ase, minimal activity with Alk ATPase, intense activity with Acid ATPase, and,

in contrast to the small B fibres, relatively low activity with SDH (figs.146-149). Such fibres thus possess a type G histochemical profile similar to that of the peripheral G fibres of SR and are in fact the tapering ends of the nuclear-bag fibres from the numerous muscle spindles scattered throughout the muscle (see chapter 17).

12.3 Ultrastructural fibre types.

The fibre types in LP corresponding to the histochemical fibre types given with SDH were identified in semi-thin (1.5μm thick) transverse Epon sections stained with toluidine blue (fig.151). Fibres were distinguished largely on the basis of diameter and mitochondrial content. Thus the largest (Large A type) fibres contain small granulations (mitochondria) that are distributed mainly around the periphery of the fibre; the medium-diameter (intermediate C type) fibres have mitochondria of greater size and density with again a tendency towards a peripheral distribution; and the smallest (small B type) fibres have mitochondria of similar size to the intermediate C type distributed evenly throughout a transverse section. All three fibre types have small, well-delineated myofibrils (see Table IV).

The appearance of the large A, intermediate C and small B fibres in low-power transverse electron micrographs (fig.153) corresponds largely to the light-microscope appearance of the semi-thin Epon sections stained with toluidine blue. Fibre types are distinguishable only by their different diameter and by the

differences in size, abundance and distribution of mitochondria. The other ultrastructural features of the fibres in transverse section are common to all three fibre types and may be outlined using the large A fibre (fig.154) as a representative example.

The small myofibrils are surrounded in the I band by single layers of tubules of sarcoplasmic reticulum, and at the A/I band junction are well-delineated by transversely-orientated tubules. They are smaller than the myofibrils of the corresponding large A fibre from the central core of SR. The A band shows only isolated tubules of sarcoplasmic reticulum and consequently the myofibrils are hardly delineated at all. Glycogen is especially abundant with granules associated with the sarcoplasmic reticulum and also located within the myofibrils of the I band. All these features are shown by the other fibre types in LP. Large A fibres (fig.154) are the largest diameter fibres and possess only sparse, small mitochondria that contain few cristae. The smaller-diameter fibre types have more abundant and larger mitochondria. Intermediate C fibres (fig.155) have mitochondria that are intermediate in size and number between those of the large A and small B fibres, and which tend to form subsarcolemmal accumulations. Small B fibres (fig.156) possess the most numerous and largest mitochondria that are distributed evenly across a transverse section.

In longitudinal section the fibre types are distinguishable only on one additional ultrastructural characteristic besides those already mentioned: the thickness of the Z line. Many features shown in longitudinal section are shared by all three fibre types.

They may be listed as follows:

(i) the Z lines have a visible substructure of Z filaments;

(ii) a prominent M line lies within a pseudo-H zone in the middle of the A band;

(iii) the transverse tubular system is well-developed and triads occur regularly at the A/I band border;

(iv) sarcoplasmic reticulum separates the myofibrils in the I band; and

(v) abundant glycogen is present in the I band, both in association with the sarcoplasmic reticulum and between the myofilaments. The large A fibres (fig.157) are characterized in longitudinal section by relatively infrequent, small mitochondria, and by straight Z lines that are the thinnest of the three extrafusal fibre types in LP. The intermediate C fibres (fig.158) have slightly larger and more numerous mitochondria, and slightly wavy Z lines of intermediate thickness between those of the large A and small B fibres. The small B fibres (fig.159) show the most numerous and largest mitochondria. The Z lines are thickest of all three fibre types and are also wavy. These features are summarized in Table IV.

12.4 Types of motor nerve ending and their distribution.

An examination of teased, silver preparations taken throughout the length of the muscle shows that the innervation of the extrafusal muscle fibres is totally by means of compact motor end-plates, as in the LP muscles of man (Dietert, 1965; Namba et al.,1968a) and

rat (Namba et al.,1968b). Multiterminal grape innervation does occur in LP, but it is confined to the nuclear-bag fibres of the muscle spindles (chapter 20). The end-plates occur throughout the muscle and are derived from medium- to large-diameter myelinated nerve fibres.

Gross examination of a LP muscle stained in toto for cholinesterase activity shows clusters of compact plate endings that occur at approximately the same level on adjacent muscle fibres (fig.82). These clusters of end-plates are scattered at intervals along the whole length of the muscle, and there is consequently no discrete, central innervation band, in contrast to the LP muscles of man and rat (Dietert, 1965; Namba et al.,1968a, b). Since teased preparations have shown only one end-plate per muscle fibre the scattered innervation suggests that the muscle fibres may not extend the full length of the muscle. The end-plates display both high acetyl-cholinesterase (AChE) and butyrylcholinesterase (BChE) activity. In addition to the end-plates, cholinesterase also stains the musculo-tendinous junctions as cholinesterase 'cuffs', and also the endings within the muscle spindles.

12.5 Ultrastructure of the extrafusal myoneural junctions.

A total of 17 extrafusal myoneural junctions were examined in transverse section under the electron microscope: 5 junctions were located on large A fibres; 7 on intermediate C fibres; and 5 on small B fibres. The basic ultrastructure of all of these endings resembles that of the typical motor end-plate of mammalian skeletal

muscle. The axon terminals overlie a sole plate and are located in synaptic grooves or gutters with extensive folding of the post-synaptic membrane. There are, however, some ultrastructural differences between the end-plates applied to the three fibre types in LP.

The neuromuscular junction of the large A fibre (figs.160-161) is composed of large and often elongated axon terminals that overlie a substantial sole-plate. The junctional folds beneath such axon terminals are numerous, long, narrow, and closely-spaced giving an extensive folded surface area. The axon terminals of the intermediate C fibre (figs.162-163) are similar to those of the large A fibre, but overlie a relatively thin sole plate. The post-synaptic folds are less closely-packed and more irregular in form. Many folds are expanded at their bases and appear bulbous, while other folds may be branched. The end-plate of the small B fibre (figs.164-165) is composed of smaller and less extensive axon terminals than those of the other fibre types, that overlie a very thin sole plate. The junctional folds are relatively widely-spaced, frequently bulbous in form, and occasionally branched. These endings on small B fibres appear to provide the least folded surface area beneath the terminals.

The axon terminals applied to the three fibre types all contain the usual organelles such as mitochondria, neurotubules and synaptic vesicles. Occasional dense-core vesicles are also present (figs.161 & 164). The characteristics of the fibre types in LP are summarized in Table IV.

Chapter 13. Hindlimb muscles.

13.1 Unidentified muscle from the hough group.

13.11 Histochemical fibre types.

This muscle was grossly pale in appearance and was selected as an example of a "fast, white, mixed muscle". The three extrafusal fibre types that compose this particular muscle (figs.166-169) are similar to the large A, intermediate C, and small B fibre types of LP (see Table V for summary). The large A fibres are the largest in diameter and are high (grey-blue) in activity with P'ase (fig.168); low ('white' pattern) with SDH (fig.169); and high (black) with Alk ATPase (fig.167). The intermediate C fibres are of medium to small diameter and are high (dark brown) in activity with P'ase; high ('intermediate' pattern with subsarcolemmal rim) with SDH; and high (grey) with Alk ATPase. The small B fibres are of small diameter and are low (yellow to light brown) with P'ase; high (red, overall pattern) with SDH; and low (minimal white) with Alk ATPase.

13.2 The distal portion of peroneus brevis (PB).

13.21 Histochemical fibre types.

The muscle fibres composing this distal, highly-modified hindlimb muscle are generally larger in diameter than the fibres of either the extraocular or the hough flexor muscles described earlier, and the difference in diameter between the largest and smallest fibres is not marked. The histochemical profile of one of the muscle fibre types in PB is also different from those of the

Table V. A summary of the light-microscope and ultrastructural characteristics of the fibre types present in sheep hindlimb and foot muscles.

Unidentified flexor from 'hough'			
Fibre types	Large A	Inter. C	Small B
Relative diameter	Large	medium	small
P'ase activity	High	High	Low
SDH activity	Low	High	High
Alk ATPase activity	High	High	Low
Peroneus brevis			
Fibre types	'conventional C'	'modified C'	B type
Relative diameter	large	medium	medium
P'ase activity	High	High	Low
SDH activity	High	High	High
Alk ATPase activity	High	Low	Low
Fibrillar pattern	——— 'Fibrillenstruktur' ———		
Innervation	——— Single plate ('en plaque') ———		
% total count	39	60	1
Myofibrils	——— small, well-delineated ———		
T system (triads)	——— regular at A/I junction ———		
M line	present	present	present
Myoneural junction	folded	folded	folded

hough flexor muscle (see Table V). In terms of oxidative and glycolytic metabolism as typified by SDH and P'ase staining there appear to be only C (oxidative/glycolytic) and B (oxidative/non-glycolytic) fibre types present in PB. Thus with SDH (fig.171) the general level of activity of nearly all the fibres is intermediate, with a 'red' pattern of fine formazan granules scattered throughout the fibre. There is no marked difference between the fibres, although the largest fibres tend to appear slightly paler, and all fibres may be regarded as relatively high in oxidative activity. With P'ase (fig.170) most fibres are high in activity (dark brown to grey-blue in colouration) with only occasional low fibres (yellow to light brown in colouration), these being the few B type fibres located among a spectrum of C type fibres. However, the 'C type' fibres of PB are unusual among the sheep muscles studied in that the high P'ase activity is not consistently matched by a relatively high level of Alk ATPase activity. Thus, while the largest of the C type fibres are high (black) for Alk ATPase (fig.172) and hence conform to the normal C type histochemical profile in sheep, many slightly smaller C type fibres are low (minimal activity). These fibres thus form a group with the unusual profile of relatively high oxidative (SDH) activity; high glycolytic (P'ase) activity; and low Alk ATPase activity, which may indicate that they are slower in contraction than the 'normal C type' fibres. In a count of 400 fibres, normal C type fibres constituted about 39%; modified C type fibres about 60%; and B type fibres about 1% of the total number. PB is not therefore, as was hoped, a typical, mixed hindlimb muscle composed of large A, intermediate C and small B fibres comparable

to those of the hough flexor muscle, and thus does not provide a completely normal control or 'baseline' muscle for comparison purposes. However, since no triple-negative type G histochemical profile is in evidence it is assumed that all the fibre types in PB are of the plate-innervated 'twitch' type.

13.22 Ultrastructure of muscle fibres.

Since the fibre types in PB are not clearly distinguished by their diameter and mitochondrial patterns they can not be categorized for ultrastructural analysis. In a transverse Epon section stained with toluidine blue (fig.152) all the muscle fibres show very small, well-delineated myofibrils with small mitochondria that are scattered evenly throughout a transverse section. In longitudinal section (fig.173) all fibres display the ultrastructural characteristics associated with 'twitch' muscle fibres (Hess, 1970): small myofibrils that are well-separated by abundant sarcoplasmic reticulum particularly in the I band; triads that occur regularly at the junction of the A and I bands; and a prominent M line that lies within a pseudo-H zone in the centre of the A band. The small mitochondria tend to lie on either side of a Z line that has Z filaments.

13.23 Extrafusal motor nerve endings.

Teased, silver preparations show that only the end-plate type of ending occurs on extrafusal muscle fibres (figs.287-288). Medium to large-diameter stem nerve fibres branch to end in twig-like,

compact endings. From each terminal twig there are usually a number of fine non-myelinated branches that terminate in small axoplasmic knobs or swellings. Such terminations make the endings less characteristically plate-like than those of SR or LP. In addition, the main nerve twig may end in a large ball ending (fig.287).

An examination of the plate endings in PB under the electron microscope shows that their fine structure is similar to that of the typical motor end-plate (fig.174-175). Axon terminals, that contain the usual organelles, lie in gutters over a substantial sole plate with extensive folding of the post-synaptic membrane (see Table V for a summary of the characteristics of the fibre types in sheep hindlimb muscles.)

13.24 Longitudinal division of muscle fibres.

Within the distal portion of PB several muscle fibres have been observed under the electron microscope to divide into two portions, although in a slightly different fashion to the longitudinal division of peripheral G fibres in SR (figs.142-143). Two such divisions of muscle fibres in PB are illustrated in transverse sections (figs.176-181). In the first example the only indication of an eventual division of the parent muscle fibre is a slight external constriction of the outer membrane (fig.176) while the internal myofibrillar pattern remains normal. A line of separation then forms between the myofibrils of the parent fibre and the myofibrils that are to eventually split off (fig.177) and the outer membrane invaginates as a thin fold along this separation line (fig.178). Just beyond the invaginating fold and around the

tip of the fold are numerous pinocytotic vesicles that line the opposed muscle membrane. Eventually the fold spreads completely across the muscle fibre and the daughter fibre splits off (fig.179) being bounded by a sarcolemma and basement membrane. In this division the daughter fibre is approximately one third of the cross-sectional area of the original parent fibre. In the second example (figs.180-181) a parent muscle fibre is shown in the process of dividing into two equal portions. In both of these examples of longitudinal division the parent muscle fibre is split for an appreciable distance along the longitudinal axis into two daughter fibres. A difference between the divisions in PB and those of the peripheral G fibres in SR is that the division in the foot muscle is towards the distal end of the muscle and not toward the belly as in SR.

It is possible that these simple longitudinal divisions of muscle fibres are the consequence of some pathological state or abnormal work load since they are associated with muscle fibres of even more unusual appearance in which the myofibrils are extensively fragmented (figs.182-183). In these occasional fibres the majority of the myofibrils appear normal with only a portion of the muscle fibre affected. The whole fragmented portion is well-separated from the parent muscle fibre, while within the portion the myofibrils are divided into irregular pieces that may be even smaller than a single myofibril. Pinocytotic vesicles are found lining some of the outer muscle membranes. Since this fragmentation occurs at the distal end of PB it is possible that only the extreme ends of the muscle

are affected.

In skeletal muscles of rat subjected to excessive work loads (Reitsma, 1970) both simple branching into daughter fibres (loc.cit., fig.5A) and also excessive fragmentation of the ends of muscle fibres (loc.cit., fig.5B) occurs. Edgerton (1970) has also observed splitting in the soleus of normal sedentary rats, but not in the plantaris or gastrocnemius. The incidence of splitting in soleus increases with amount of exercise. Thus, although a pathological cause for fibre division in PB cannot be ruled out (see e.g. Bell & Conen, 1968), there is evidence to suggest that division of muscle fibres is a normal occurence in muscles that have a postural function and which may be subject to heavy work loads.

Appendix 1. Parasitic infection.

One minor feature common to both SR and LP muscles of sheep is the high incidence of infection by the protozoan Sarcocystis, a muscle parasite widespread in the animal kingdom. Under the light microscope the organisms appear as sausage-shaped cysts about 250μm in length and 100μm wide, longitudinally orientated within a single muscle fibre (fig.184). At higher magnification (figs.185-186) the cyst wall that separates the parasite from the thin peripheral rim of myofibrils is apparent. The complex layered structure of the wall may be due to calcification. Whereas under the light microscope the central spores appear to be uniform with no apparent trabeculae, higher magnification shows that in fact the spores occur in groups separated by fine trabeculae.

SECTION C. DISCUSSION

The two layers present in the belly of sheep SR, a central core of mainly large-diameter muscle fibres and an orbital rim of small muscle fibres, correspond to those first observed in sheep extraocular muscle by Voss (1935). In fact the peripheral location of small 'red' fibres, high in oxidative enzyme activity, is a constant feature in the structural organization of extraocular muscles, not only in mammals (1.1), but also in fish (Kilarski, 1965; 1966; 1967a, b; Kilarski & Bigaj, 1969; Kordylewski, 1974) Amphibia (Kilarski & Bigaj, 1969; Nowogrodska-Zagorska, 1974), reptiles (Kaczmarski, 1969) and birds (Kaczmarski, 1970b; Alvarado-Mallart, 1972; Maier, Eldred & Edgerton, 1972). There appears to be a trend for the layers to become less distinct as one passes from fish to mammals. For example, in the eye muscles of the goldfish (Kilarski & Bigaj, 1969) the layers appear 'like separate muscles glued together', whereas in dog, cat and goat (Kato, 1938) transition layers of mixed fibre types are present. It is interesting that a similar layered pattern is also evident in the cat tensor tympani (Asmussen & Wohlrab, 1971), one of the few other mammalian muscles in which physiologically slow, grape-innervated muscle fibres have been reported (Erulkar, Shelanski, Whitsel & Ogle, 1964; Fernand & Hess, 1969).

It is tempting to speculate that the layered organization in extraocular and certain other mammalian muscles is an indication of their phylogenetically ancient status. In elasmobranch fish (Bone, 1966) each myotome is composed of a thick inner layer of large

'white' fibres covered by a thin outer layer of small 'red' ones, an arrangement comparable to that in eye muscles. In the dogfish the peripheral 'red' fibres are slow and are used during sustained activity such as basal cruising, while the central 'white' fibres are fast and come into play only when vigorous movement is required. Such a dual functional system is possible in an aquatic environment where the problem of supporting the body weight against gravity is not acute. One consequence of the evolutionary emergence of fish onto land has been the reduction of the large muscle mass, and this has been achieved largely at the expense of the peripheral, 'red', grape-innervated component. In frog gastrocnemius (Engel & Irwin, 1967), for example, the grape-innervated fibres are restricted to a small superficial patch and constitute less than 1% of the total fibre content. In mammalian skeletal muscles the grape component has almost completely disappeared except in the isolated cases of the rectus and oblique extraocular muscles (1.2), the tensor tympani and stapedius muscles of the ear (Erulkar et al.,1964; Fernand & Hess, 1969), and the oesophageal muscles (Comline & Message, 1965; Floyd, 1971). The grape innervation in these particular muscles is presumably an inheritance from the original primitive pattern. That the proportions of grape-innervated fibres in these muscles is very low is possibly indicative of their vestigial status.

If the layering in extraocular muscles does in fact reflect a primitive condition, one might also expect some of the primitive functional differences between central core and orbital rim layers to be retained. For example, the orbital rim could be responsible for slow fixation eye movements, while the central core carries out

faster pursuit movements.

At the origin and insertion ends of sheep SR there is also a peripheral patch layer, which, although composed mainly of grape-innervated muscle fibres, is not predominantly 'red' in character. This layer corresponds to the thin sheet of 'white', grape-innervated muscle fibres that lie on the outer surface of the dogfish myotome immediately underneath the skin. There are several possible reasons why similar proximal and distal peripheral patches have not been identified in the extraocular muscles of other mammalian species. First, the patches may be present only in some of the rectus and oblique muscles. Second, because of the thinness of the patch layers and their peripheral location, it is easy to overlook them when present, or to lose them completely when cutting frozen sections. Finally, if the patch layers are present only at each end of a particular extraocular muscle, they would probably be missed in randomly-sampled transverse sections.

However, in terms of innervation there is not an exact parallel between the layered extraocular muscles and the layered dogfish myotome. In the dogfish the central 'white' layer is entirely 'plate-innervated', each fibre receiving a basket ending at one myoseptal end (Bone, 1966), whereas the fibres composing the peripheral 'red' layer and the superficial 'white' layer receive distributed grape endings. In sheep SR grape innervation is supplied to about 7% of the largest-diameter fibres of the central core, about a third of the thinnest fibres of the orbital rim, and to the greater proportion of intermediate-diameter fibres in the peripheral patches. Although grape endings are thus most frequent in the peripheral layers, they are

present throughout the muscle, as occurs in the extraocular muscles of other mammalian species (1,2), and they are not located on muscle fibres that are 'red' in character.

It has been further shown (Barker & Harker, 1972) that the grape innervation in sheep SR is of two types: a 'compact' type located on the 'Felderstruktur' fibres of the central core; and an 'extensive' type located peripherally. These two types of grape ending may correspond to the 'extensive' and 'compact' grape endings in the extraocular muscles of rabbit (Cheng-Minoda et al.,1968) and rat (Teräväinen, 1968a), even though these endings occasionally occur on the same muscle fibre (Teräväinen, 1968a).

Since there is no equivalent grape-innervated fibre type in the central 'white' layer of a dogfish myotome, the derivation and functional significance of a distinct central core G-fibre type in sheep SR is problematical.

There are other suggestions in the literature, apart from those already mentioned (1,2), that there are two distinct patterns of grape innervation in extraocular muscles. The illustrations of grape endings in goat eye muscle (Silver, 1963; plate 3, figs.e & f) show them to be comparable to the compact grape endings on the central core G fibres of sheep. On the other hand the extensive grape terminations in the eye muscles of the guinea pig (Hess, 1961a; fig.14) obviously occur on neighbouring fibres, as occurs in the peripheral patch layers of sheep SR, and they show a similar profuse pattern of innervation.

The extensive grape innervation on the peripheral G fibres

in sheep SR appears unique to extraocular muscles. Zenker & Gruber (1967) have compared the ratio of overall synaptic area to muscle-fibre volume in a variety of grape-innervated muscle fibres. The ratio was lowest in pigeon gastrocnemius, frog dorsalis scapulae, and chicken anterior latissimus dorsi muscles; much greater in the extraocular muscles of tench, rhesus monkey and man; and greater still in the eye muscles of the finch, hen and owl. It might be expected that such a profuse grape supply would obviate the need for the respective muscle fibres to propagate any impulses, since no part of the fibre is far removed from a synaptic region. That two types of excitatory post-synaptic potential are seen in rabbit slow fibres (Matyushkin & Drabkina, 1970) might conceivably be the consequence either of different sizes of synaptic areas derived from the same grape axon, or polyneuronal grape innervation of a muscle fibre by more than one axon. In the situation where neighbouring innervation zones are supplied by separate axons Peachey (1971) has discussed the possibility that impulses from the two zones, if sufficiently close, could summate to exceed threshold for an action potential. A similar situation presumably operates in teleost twitch muscles that are grape-innervated (see Barker, 1968). In tench these respond to single shocks of low intensity with local potentials and weak contractions, but with a stimulus of increased intensity give diphasic propagated potentials (but no overshoot) and stronger twitches.

The pattern of grape innervation on the central core G fibres of sheep SR is similar to that on the muscle fibres in the anterior latissimus dorsi of the chicken (Hess, 1961b) and the domestic hen (Silver, 1963), in which the compact innervation zones are regularly spaced apart (Hess, 1961b; Zenker & Gruber, 1967). In the case of

the adult chicken (Hess, 1961b) the endings are separated by distances of about 1,000μm. The individual innervation zones on the central core G fibres are also similar in form to those on the slow fibres in frog iliofibularis tonus bundle (Hess, 1960). The pattern of innervation in frog, however, is extremely irregular. The endings may be as close as 60-120μm or separated by distances of 1mm or more. Whereas frog slow fibres do not propagate, the grape-innervated fibres in chicken (Ginsborg, 1960) respond in vitro to a single stimulus with a propagated action potential followed by a slow twitch. It is not known whether this feature is related to the regular pattern of grape innervation in avian muscles.

Because of the presence of junctional folds, albeit sparse and irregular in form, beneath the axon terminals of both types of grape ending in sheep SR, they cannot be directly compared to the classic grape endings of frog (Page, 1965). The observation that in rat extraocular muscles weakly-developed folding is present only beneath the extensive grape endings of the periphery (Mayr, 1971) is not true of sheep eye muscles.

The junctional folding beneath the plate terminals in sheep SR is more extensive than that shown under the plate endings in cat (Pilar & Hess, 1966; Hess, 1967) and albino mouse (Saltpeter et al., 1974) extraocular muscles, and is more variable in form. Whereas the end-plates on the large A fibres have the most regular and closely-spaced junctional folds, the folding in the intermediate C fibre is sparser, and in the small C fibres is the least frequent. The end-plates in the soleus of the mouse (Duchen, 1971) have less numerous and shorter junctional folds than those in the gastrocnemius, and the degree of development of folding is thus related to the speed of

contraction of whole muscles. If a similar relationship holds for individual muscle fibre types within the same muscle it would suggest that the large A and intermediate C fibre types in sheep SR are faster-contracting than the small C fibres.

With regard to the morphological fibre types present in sheep SR, the large G fibres of the central core and the small/intermediate G fibres of the peripheral layers both show many of the features characteristic of slow muscle fibres (Hess, 1967; 1970). Both exhibit sparse and irregular T-systems, reduced amounts of sarcoplasmic reticulum, irregular size and disposition of myofibrils ('Felderstruktur'), and mainly smooth myoneural junctions. However, the central core G fibres resemble the grape-innervated fibres in the anterior latissimus dorsi of the chicken (Hess, 1961b; 1967; Mayr, 1967) in that they possess prominent M lines, whereas the peripheral G fibres resemble the slow fibres of the frog (Peachey & Huxley, 1962; Page, 1965) and the garter snake (Hess, 1965) in that an M line is lacking. If the central and peripheral grape-innervated fibres in the extraocular muscles of other mammalian species exhibit a similar variability in M-line morphology, this could explain some of the discrepant reports in the literature (1.3).

The large A, intermediate C and small C fibres in sheep SR all have a 'twitch-type' morphology (Hess, 1967). They have regular T-systems, relatively abundant sarcoplasmic reticulum, well-delineated myofibrils ('Fibrillenstruktur'), M lines, and folded myoneural junctions, although each fibre type varies with regard to mitochondrial content. In the possession of an M line the 'Fibrillenstruktur' fibres of sheep SR resemble those in the inferior oblique of the cat (Peachey,

1968), whereas in the rhesus monkey (Cheng & Breinin, 1966; Miller, 1967) and rat (Mayr, 1971) an M line is present only in the mitochondrial-poor (white A type) 'Fibrillenstruktur' fibres. The mitochondrial-rich (red C type) fibres in rhesus monkey and rat have a comparable ultrastructure to plate-innervated fibres in the soleus of albino mouse (Duchen, 1971) that also lack M lines, and it is possible that they are similarly slow-contracting.

The several morphological fibre types in sheep SR can each be equated with similar fibre types in the extraocular muscles of other species, if M line characteristics are ignored. In a review of the most comprehensive morphological studies, Peachey (1971) considers that five distinct fibre types are present in extraocular muscles, of which two occur in the orbital rim and three in the central core. The present study has extended this scheme to include six fibre types, some of which are present in more than one layer of the muscle.

The six fibre types in sheep SR were in fact initially distinguished in terms of their histochemical profiles and diameters as large G, large A, intermediate C, small C, small G and intermediate G types. Of these the intermediate G type is exclusive to the peripheral patch layer, which is not described for any other species. It is therefore possible to compare only the findings for the orbital rim and central core layers with the results from other studies, such as those of Miller (1967) on rhesus monkey; Asmussen et al. (1970; 1971) on rat, cat, rabbit and guinea pig; and Mayr (1971) on rat. There appears to be general agreement among these authors about the pattern of organization in the central core layer. Miller (1967)

distinguishes three fibre types that exhibit A, C and G-type histochemical profiles, respectively. Asmussen et al. (1970; 1971) characterized a further C-type fibre in their total of four types in the central core. The histochemical profiles of these fibre types closely monitor those of the large G, large A, intermediate C and small C fibres of sheep SR. For the orbital rim layer Miller (1967) and Asmussen et al. (1970; 1971) both describe two fibre types. These exhibit C-type profiles and appear equivalent to the intermediate C and small C fibre types in the orbital rim of sheep SR. In addition, Mayr (1971) describes a minority of thin fibres in this layer that exhibit very low SDH activity. These correspond to the small G fibre types in sheep SR. Mayr (1971), Miller (1967) and Asmussen et al. (1970; 1971) consider the orbital rim and central core C-type fibres as separate types. In the case of sheep SR, the differences between central and peripheral C-type fibres were not considered sufficiently significant to warrant such a classification.

The part that each fibre type plays in the movement of the eye remains conjectural, since it has not yet proved possible to correlate the morphological properties of individual fibre types in extraocular muscles with their functional characteristics. Such a correlation has been made, however, in amphibian hindlimb muscles such as iliofibularis (see Smith, Blinston & Ovalle, 1973 for review) that have a complex layered organization and which could well be analogues of mammalian extraocular muscles. The 'zoned' muscles in frogs and toads contain five fibre types, three of which are considered to be fast and two of which are thought to be slow. In terms of ultrastructure, relative diameter and histochemical profile the three plate-

innervated fibre types in sheep SR: the large A, intermediate C and small C types, may be broadly compared with the three fast fibre types in amphibia (Engel & Irwin, 1967; Smith & Ovalle, 1973) that are respectively listed as types 1, 2 and 3 by Smith et al. (1973).

The histochemical profiles of the peripheral G and the central G fibre types in sheep SR respectively resemble those of the type 4 and type 5 fibres in frog and toad (Smith et al.,1973). Both types of slow fibre in toad are located in the iliofibularis tonus bundle (Lännergren & Smith, 1966), but whereas in Xenopus laevis the slow fibres are thin ,in Bufo viridis slow fibres (type 5?) are among the largest in the muscle and thus parallel the size and central location of the large G fibres in sheep. In the proximal portions of the zoned muscles of frog the slow fibres (type 4?) have a superficial location (Engel & Irwin, 1967) and are associated with thin mitochondrial-rich muscle fibres (Asmussen & Kiessling, 1970). Such an arrangement is reminiscent of the peripheral patch layer in sheep.

A study of the isometric contractions of single motor units in toad muscles (Smith & Lännergren, 1968; Smith et al.,1973) has indicated that five distinct kinds of motor unit are present that correspond to the five types of muscle fibres. Type 1 motor units (≡ large A fibres in sheep SR?) have the shortest twitch contraction times, a high twitch-tetanus ratio, but little resistance to fatigue. Type 2 motor units (≡ intermediate C fibres?) have intermediate twitch contraction times, lower twitch-tetanus ratios, and are less susceptible to fatigue. The type 3 units produce 'small twitch responses' to single stimuli and have very low twitch-tetanus ratios.

Smith et al. (1973) consider these units to be composed of muscle fibres that Ork?and (1963) described as not responding to a single stimulus with a propagated action potential. The type 4 (≡ peripheral G fibres?) and type 5 (≡ central G fibres?) units are both slow and respond to repetitive stimulation in a graded fashion to produce small tensions. The type 4 units develop tension much more rapidly than do the type 5 units.

If the physiological characteristics of the fibre types in mammalian extraocular muscles are similar to those of the comparable fibre types in Amphibia, the functional properties of the various fibres in sheep SR would be as follows. The peripheral patches contain mainly intermediate G fibres that would be slow in function. The orbital rim is composed of a minority of small G fibres that would also be slow, together with small C and intermediate C fibres that are probably resistant to fatigue and are slow- and moderately fast-contracting, respectively. The central core contains slightly less than half of large A fibres that are likely to be fast-contracting, but easily fatigued, about half of fatigue-resistant small C and intermediate C fibres like those of the orbital rim, and a small proportion of large G fibres that are probably the slowest in the muscle. From such considerations it would appear that the main functional role of the peripheral patch and orbital rim layers is related to slow, small and maintained eye movements, whereas the central core is concerned more with faster, larger and more sporadic types of movement. Such a dual functional system has already been predicted from a comparison of the layering pattern in extraocular muscles with that of a fish myotome. The systems in eye muscles are

not totally segregated, as in fish, since there are also present in the central core of SR small C and large G fibres, both of which are probably slow-contracting. The functional significance of the central G fibres in extraocular muscles is particularly puzzling. In the rat, guinea pig, albino mouse and man they may be present only in specific rectus and oblique muscles (Siebeck & Krüger, 1955). When the central G fibres are present they have a scattered distribution, but are not generally the largest fibres in the muscle, as in sheep SR. They constitute only a small proportion of the fibres in the central core and their contribution to total muscle tension is likely to be small. In sheep SR it is not known whether the central core G fibres, unlike the surrounding plate-innervated fibres, stretch the full length of the muscle. Their large diameter and uniform distribution suggests that they might have a relatively inert structural function, acting to give a measure of cohesiveness to the movement of the central layer.

The question arises as to how the rectus and oblique muscles come to be organized into distinct layers each with different populations of muscle fibres. Several possibilities are conceivable that involve the inductive potential of either the developing muscle fibres or the entering motoneurons, or even other factors such as the differing density of blood supply within a muscle (see Maier, Eldred & Edgerton, 1972). That some influence is exerted by motoneurons on the distribution of histochemical fibre types in extraocular muscle has recently been shown in the baboon (Durston, 1974). The most notable effects of re-innervation following oculomotor nerve section are an increase in the number of fibres that are low in Alk ATPase activity (= G type fibres?) and their clumping together to

give muscle-fibre groups of one histochemical type. Durston suggests that the motor axons which normally supply these fibres (grape axons?) regenerate more quickly or sprout more readily and so re-innervate additional fibres, imposing on them new histochemical characteristics.

Is there any evidence from the known physiology of extraocular muscles to support the hypothetical functional scheme described for the numerous fibre types in sheep SR? Unfortunately, only in few physiological investigations has the layered organization of eye muscles been taken into account and some correlation of physiological and morphological properties made. For example, according to Kern (1965) the orbital rim layer in rabbit extraocular muscle is composed almost exclusively of thin 'Felderstruktur' fibres that give a well-maintained contracture on exposure to acetylcholine. Although it is unlikely that all the orbital rim fibres of rabbit are grape-innervated slow fibres, at least some of them resemble frog slow fibres in their pharmacological properties, these presumably being equivalent to the peripheral G fibres of sheep. It is surprising that no contracture was given by the central core layer in rabbit since a small proportion of grape-innervated fibres are probably present.

In cat extraocular muscles extracellular recording during stimulation of the III nerve produces action potentials that are either prolonged monophasic units or short biphasic spikes (Nemet & Miller, 1968). Whereas both types of response may be recorded from the central core and orbital rim layers, biphasic spikes predominate in the central core and monophasic units in the orbital rim. These monophasic units are considered by Nemet & Miller to represent slow-twitch muscle fibres, supplied by thickly-myelinated axons, that probably

correspond to the small mitochondrial-rich fibres distributed mainly within the orbital rim layer, but also in the central core. The similar small C fibres in sheep have been inferred in the previous discussion to be slow-contracting and fatigue-resistant. Their electrical properties may be unusual in that they do not propagate action potentials in response to a single stimulus (cf. Orkland, 1963).

There still remains the controversial point over the propagatory or non-propagatory nature of the grape-innervated components of cat extraocular muscle. Peachey (1968) resolves this by assuming that Hess & Pilar (1963) and Bach-y-Rita & Ito (1966a) studied different types of grape-innervated fibre. He identifies the relatively thick 'Felderstruktur' fibres that possess an M line and are comparatively numerous in the superior oblique as the ones shown by Hess & Pilar (1963) and Pilar (1967) to possess typical slow properties. These fibres correspond to the central core G fibres in sheep, which have been predicted to be the slowest fibres in the muscle. The fibres studied by Bach-y-Rita & Ito (1966a) in the superior rectus and inferior oblique muscles of the cat are located in the orbital rim layer and may possibly be equivalent to the small G fibres in sheep SR. However, Bach-y-Rita & Ito suggest that all the orbital rim fibres are "slow multi-innervated twitch" fibres and that they total approximately one third of all the fibres in the muscles studied. Such figures do not accord with the available morphological and histochemical data. In rat (Mayr, 1971) and sheep (Harker, 1972a) extraocular muscles the orbital rim contains only 20-30% of grape-innervated fibres. It therefore remains a possibility

that Bach-y-Rita & Ito recorded, at least in some cases, from small C-type fibres that are plate-innervated, but may have unusual slow-twitch properties. This may explain the apparently conflicting results concerning the ability to propagate of the peripheral "slow" component. Two slow-contracting systems may operate in the orbital rim, one of which may propagate and another that does not.

One would expect, if morphological and histochemical fibre-typing in extraocular muscles is meaningful, that several distinct types of motor unit are present in eye muscles, each composed of muscle fibres with distinct electrical properties.

The formerly held view that the various types of eye movement are each the function of one type of motor unit and thus of a distinct fibre type (see e.g. Jampel, 1967) has recently been challenged. Studies in which the activity of single nerve cells and fibres in the brains of alert monkeys has been recorded (Fuchs & Luschei, 1970; Robinson, 1970; Schiller, 1970; Keller & Robinson, 1972) have suggested that all cells in the oculomotor neuronal pools participate in the same way in each type of eye movement, whether it be saccadic, pursuit, fixation, vergence, or vestibular. The discharge rates of the cells are dependent on eye velocity as well as position. This supports the view that different muscle fibre types cannot be simply correlated with types of eye movement, even with regard to the fast and slow motor systems. However, according to threshold for stimulation there are two populations of neurones in the oculomotor units of monkeys. 'Low-threshold' fibres are likely to be the first to be recruited during a contraction and they probably correspond to C-type fibres that are relatively resistant to fatigue.

Such fibres may be active in fixation movements that are constantly present during waking hours. 'High-threshold' fibres may be brought into play only as the contraction increases, and they probably correspond to the A-type fibres that are fast-contracting, but susceptible to fatigue. These fibres are likely to act mainly during saccades in the 'on' direction. The point of recruitment of the slow fibres in such a system is difficult to infer. It is even possible that in terms of eye movement in mammals the slow components are vestigial.

In that the levator palpebrae (LP) of sheep does not show the pronounced layering characteristic of the rectus and oblique muscles, and contains only plate-innervated extrafusal muscle fibres, it conforms to the pattern shown by the LP muscles of other mammalian species (3.2). The ultrastructural characteristics of all the extrafusal muscle fibres in sheep LP correspond to those observed in 'twitch' type fibres (Hess, 1967) that exhibit small well-delineated myofibrils, abundant sarcoplasmic reticulum, distinct M lines, regular triads, and postjunctional folds beneath the nerve terminals. The fine structure of the large A, intermediate C and small B fibres and their respective end-plates shows resemblances to the 'white', 'red' and 'intermediate' fibres in, for example, rat skeletal muscle (see e.g. Gauthier, 1971; Ogata et al.,1967). The variation in the extent of the junctional folding between the plate endings of each fibre type in sheep LP may directly reflect the different speeds of contraction of the muscle fibre types.

By analogy with the large A and intermediate C fibre types in sheep SR, the similar fibre types in LP might both be expected to

be fast-contracting, but susceptible and resistant to fatigue, respectively. The small B fibre type in sheep LP has a similar histochemical profile to the 'intermediate' fibre of rat and guinea-pig skeletal muscle (Edgerton & Simpson, 1969). This fibre type in cat gastrocnemius has recently been shown to be slow-contracting and extremely resistant to fatigue (Burke, Levine, Zajac, Tsairis & Engel, 1971; 1973). If the small B fibre in sheep LP has similar properties, then functionally it would appear to have replaced the small C and G fibre types that are present in SR. From this reasoning the main capacity of sheep LP would be for fast contraction, using either the large A or intermediate C fibres that total about 75% of the fibres in the muscle. To a lesser extent LP would be capable of maintained activity because of the intermediate C and small B fibres that constitute about half of the fibres. It must be borne in mind, however, that the retractor bulbi muscle of the cat, although containing three histochemical fibre types (Asmussen et al., 1971), shows only a uniform population of 'fast-twitch' muscle fibres in terms of electrical properties (Bach-y-Rita & Ito, 1965). Since the LP muscle works with SR so that upward movement of the eyeball is paralleled by a raising of the lid with subsequent maintainance of position, it is likely that LP may be more heterogeneous in its physiological properties than RB, which has the protective function of intermittently but rapidly retracting the globe.

Of the control skeletal muscles examined in sheep, only the hough flexor muscle conformed in its histochemical staining pattern to that commonly reported for 'fast, white, mixed' mammalian skeletal muscles (see chapter 4). Large A, intermediate C and small B fibre

types are present with profiles that resemble those of the similarly-named fibres in sheep LP. Consequently the level of activity with Alk ATPase approximates to that shown with P'ase.

The distal portion of peroneus brevis (PB) differs in several respects from either the LP or hough muscles. First, there is little variation in either the diameter or the oxidative enzyme activity of the three fibres present. Second, the percentage of B fibre types is negligible, being of the order of 1%. Third, although the two other fibre types both possess C-type histochemical profiles in terms of oxidative and glycolytic metabolism, one type ('conventional' C type) shows high Alk ATPase activity whereas the other type ('modified' C type) does not. If Alk ATPase is assumed to monitor intrinsic speed of contraction then the 'modified' C type is presumably slow-contracting and may take on the functions normally carried out by the B-type fibres. A similar series of histochemical profiles to those shown in PB are exhibited in the sheep diaphragm (Davies & Gunn, 1972), which is composed of 43% 'conventional' C-type fibres, 43% 'modified' C-type fibres, and 14% B-type fibres. As in sheep PB, the majority of diaphragm fibres have a high capacity for combined glycolytic and oxidative metabolism, and a minority have a capacity for oxidative metabolism only. It would appear that sheep PB muscle is primarily postural in function and adapted for slow but maintained contraction. As such it is perhaps not typical of sheep hindlimb musculature, although it serves as an additional control muscle since all the muscle fibres are plate-innervated and have an ultra-structure typical of 'twitch' fibres (Hess, 1967).

PART III - RECEPTORS IN EXTRAOCULAR MUSCLES

SECTION A. REVIEW OF BACKGROUND INFORMATION

Chapter 14. Structural review.

The structure of receptors found in extraocular muscles has been reviewed by Cooper, Daniel & Whitteridge (1955), Hosokawa (1961), and, recently, by Barker (1974). In addition to muscle spindles and tendon organs, extraocular muscles may contain a variety of receptors. These include palisade endings in the musculo-tendinous regions, sensory endings on extrafusal muscle fibres, and various endings in intramuscular connective tissue and tendon.

14.1 Muscle spindles.

'Spindle-like' structures in eye muscles were first described by Siemerling (1888) in an atrophic human inferior rectus muscle. Recognizable muscle spindles were subsequently observed in the eye muscles of man (Crevatin, 1901; Schiefferdecker, 1904; Buzzard, 1907) and in an extensive range of artiodactyls including the ox (Crevatin, 1900; Dogiel, 1906) the sheep, goat, deer and wild pig (Cilimbaris, 1910), the embryonic domestic pig (Sutton, 1915), the calf (Wohlfart, 1935), and the giraffe, cow, pig and gnu (Cooper & Daniel, 1949). Spindles have also been found in the eye muscles of the chimpanzee (Cooper & Daniel, 1949), the horse (Bonavolonta, a1956a), the albino mouse (Mahran & Sakla, 1965) and the macaque monkey (Greene & Jampel, 1966).

Muscle spindles have been reported absent from the eye muscles of several mammals including cat and rabbit (Sherrington, 1897), the dog, fox, hare and rat (Cilimbaris, 1910), the cheetah and bear (Cooper & Daniel, 1949), and the squirrel monkey (Ito & Bach-y-Rita, 1969). Spindles are also absent from the eye muscles of several avian species (Maier, de Santis & Eldred, 1971). This list should not be taken as final since several negative reports have subsequently proved incorrect. For example, the eye muscles of the domestic pig and horse were initially reported to lack spindles (Cilimbaris, 1910), but later reports on the pig (Sutton, 1915; Cooper & Daniel, 1949; Bonavolonta, 1958) and horse (Bonovolonta, 1956a) showed them to be present. Similarly, spindles were finally detected in the eye muscles of the macaque monkey (Greene & Jampel, 1966) after unsuccessful searches by Sherrington (1897) and Cooper & Daniel (1949). They proved to be absent from the oblique muscles and present up to a maximum number of six in the rectus muscles, mainly near the origin. The most surprising example, however, concerns the presence of spindles in the eye muscles of man. Whereas many workers (e.g. Batten, 1897; Baum, 1900) searched in vain for spindles, and other authors (Crevatin, 1901; Buzzard, 1907) stressed that spindles are present but extremely scarce, the recent reports of Sunderland (1949), Cooper & Daniel (1949), Merrillees, Sunderland & Hayhow (1950), Voss (1957) and Inoue (1958) indicates that from 20-70 spindles may be present in each of the rectus and oblique muscles.

The spindles of extraocular muscles, particularly those of man, differ in several respects from the typical mammalian limb

muscle spindle (see reviews by Matthews, 1972; Barker, 1974).

In the rectus and oblique muscles of man the spindles are much shorter than those of other somatic muscles (Merrillees et al., 1950; Cooper & Daniel, 1949; Inoue, 1958). There are usually four intrafusal muscle fibres, within a range of 1-15 (Merrillees et al., 1950), and these are enclosed at the equator by a remarkably thin capsule composed of only 2-3 lamellae. Equatorial nucleation is inconspicuous and the sensory endings are apparently all of a similar 'simple' type (Cooper & Daniel, 1949). The 20-70 spindles that are present in each human eye muscle are restricted in their distribution to the proximal and distal portions, within a peripheral rim of thin muscle fibres (Cooper & Daniel, 1949; Merrillees et al., 1950; Voss, 1957; Inoue, 1958). The diameter of the intrafusal and surrounding extrafusal muscle fibres is similar (Cooper & Daniel, 1949; Mukuno & Nomura, 1969), and this feature, together with the thinness of the capsule, makes the spindles relatively inconspicuous. The spindles in the eye muscles of man have, in fact, been likened to 'primitive foetal spindles' (Cooper & Daniel, 1949).

The spindles in the eye muscles of the macaque monkey (Greene & Jampel, 1966) exhibit similar features to those of man. They are small in size, contain from 2-8 intrafusal fibres, and have extremely thin capsules. The few spindles present are located mainly in the origin portions of the muscles among the small muscle fibres of the outer rim.

The extraocular muscle spindles of the albino mouse (Mahran & Sakla, 1965) contain from 2-7 intrafusal fibres and also have thin capsules. They are more numerous than in the macaque monkey, with up to five spindles visible in any high-power field.

By contrast the extraocular muscle spindles of the artiodactyls are less simple in form and are extremely numerous. Bonavolonta (1956a) has shown that the eye muscle spindles of horse, ox and sheep are more complex than those of man. Cooper, Daniel & Whitteridge (1951) counted 120 spindles in an inferior oblique muscle of the goat, and Cilimbaris (1910) found 281 spindles in a sheep lateral rectus. In these animals the spindles are scattered throughout the length of the muscles and are located peripherally, often on the inner border of the peripheral rim of thin muscle fibres (Cilimbaris, 1910; Cooper et al., 1951). In the rectus muscles of the sheep (Cilimbaris, 1910) the spindles are largely absent from the portion that lies adjacent to the eyeball, whereas in the oblique muscles, where the transverse section is circular, the spindles are distributed around the whole periphery. In sheep and goat eye muscle spindles intrafusal fibres are present that contain aggregations of nuclei beneath the annulo-spiral sensory nerve endings (Cilimbaris, 1910; Cooper et al., 1955). Such nuclear-bags with transitional myotube regions were first described by Cilimbaris (1910) who called them bläschenförmige Gebilde. He proposed that their function was for the 'refinement of receptivity of the elaborate nerve apparatus' (translated from the German, page 741).

Sheep eye muscle spindles may contain from 3-15 intrafusal

muscle fibres (Cilimbaris, 1910), those of goat from 2-12 (Cooper et al., 1951). The intrafusal fibres are usually much smaller than the surrounding extrafusal ones, and the capsule is more substantial than in man. In sheep Cilimbaris (1910) reported a capsule composed of up to 12 lamellae, although Scalzi & Price (1970) showed a maximum of only 5 in an ultrastructural investigation.

The illustrations of the sensory endings in sheep eye muscle spindles (Cilimbaris, 1910) show that 'annulo-spiral' and 'flower-spray' endings are present (although they are not described in those terms) together with intermediate forms. Comparable endings have been described by Denny-Brown (1928) in sheep and by Cooper et al. (1955) in sheep and goat. The motor innervation of sheep eye muscle spindles was found to consist of 'plate' endings, usually located in the juxta-equatorial region (Cilimbaris, 1910). According to Cilimbaris, sheep eye muscle spindles show a number of unusual features. First they vary enormously in length, ranging from singly-encapsulated spindles 0.05mm long, to 'multibellied' types up to 12.4mm in length that have a tandem series of encapsulated areas. Second, the parent intrafusal fibres that enter a spindle at one pole divide dichotomously to form a network. This network becomes re-organized towards the other pole into parent fibres that may differ in number from those entering the first pole. Third, muscle fibres may also enter spindles via the capsule instead of at the pole. Cilimbaris calls these fibres Zwischenpolfasern. Finally, Cilimbaris also records having seen a single muscle fibre with a thin connective tissue capsule, but lacking any innervation (see pages 716-717).

The muscle spindles in the rectus and oblique eye muscles of sheep are surrounded by both plate-innervated 'twitch' extrafusal muscle fibres and grape-innervated 'slow' fibres, a condition commonly found in the muscles of lower vertebrates, but comparatively rare in mammals. The question of how such sheep spindles receive their motor innervation has been recently investigated by Barker & Purdy (quoted in Barker, 1968). They have compared the nature of the fusimotor innervation of rectus and oblique muscles with that of retractor bulbi, levator palpebrae, and small foot muscles. It was shown that in the rectus and oblique muscles the spindles receive collateral plate and collateral grape innervation via branches from the extrafusal 'twitch' and 'slow' motor systems, respectively. The collateral innervation from the grape axons is particularly striking and easy to demonstrate, and was in fact illustrated by Cilimbaris (1910, see his fig.10) although he did not describe it in these terms. The spindles in the retractor bulbi and levator palpebrae muscles receive a collateral plate innervation and a grape innervation that is specifically fusimotor. The spindles in the foot muscles studied are innervated in a similar fashion to cat hindlimb muscle spindles (Barker, 1968; 1974) and receive fusimotor plate and trail endings.

14.2 Sensory endings on extrafusal muscle fibres.

Other sensory endings in extraocular muscles resemble the 'annulo-spiral' and 'flower-spray' endings of the spindle, but are found on otherwise normal extrafusal muscle fibres. Dogiel(1906)

first illustrated such endings in the eye muscles of the horse. In his account of grape innervation he includes a description of single extrafusal muscle fibres with complex spiral nerve endings. Similar endings were subsequently reported in the eye muscles of rhesus monkey (Tozer & Sherrington, 1910), rabbit (Hines, 1931), cat (Pallot, 1934; Cooper & Daniel, 1949), man (Hirano, 1941; Daniel, 1946; Wolter, 1955; Okamoto, 1957; Sas & Appeltauer, 1963), cow (Sampaolo, 1952), mangabey monkey (Cooper & Fillenz, 1955) and albino mouse (Mahran & Sakla, 1965). Of these reports that of Daniel (1946) on human extraocular material is the most detailed. He divides the spiral endings into simple forms that comprise 3-8 spirals around muscle fibres of small diameter, and complex forms that are more common and which supply muscle fibres of medium to large diameter. The endings are fairly abundant, especially in the belly of the muscle where up to one third of the muscle fibres are supplied. Daniel postulates that the spiral endings constitute extremely simplified muscle spindles.

The slightly more complex encapsulated endings described by Sas & Appeltauer (1963) in the eye muscles of man are intermediate in form between the spiral endings of Daniel and classical muscle spindles. In these 'atypical spindles' the spiral endings on a single muscle fibre are enclosed within one or several fusiform capsules in series, and as such bear some resemblances to the monofibral spindles of snakes and lizards.

14.3 Receptors in the musculo-tendinous region.

Tendon organs and palisade endings both occur in the musculo-tendinous region of eye muscles, and this has led to some confusion in the literature.

14.31 Tendon organs.

Tendon organs were first described by Golgi (1878; 1880) who called them 'musculo-tendinous end-organs'. Golgi found tendon organs in a wide range of muscles from higher vertebrates, but stated that they were absent from eye muscles. Several illustrations of tendon organs in the extraocular muscles of man, ox and pig were, however, published in 1890 by Ciaccio. Marchi (1892) confirmed these findings and showed that each eye muscle of the ox contains from 4-6 tendon organs. "Similar endings" were present in the eye muscles of pig, rabbit, dog and man, where they were found only with difficulty. However, Dogiel (1906), in his study of the eye muscles of man, monkey, horse, ox, dog and cat, found typical tendon organs only in the ox. More recently the presence of tendon organs in eye muscles has been reported for the horse, sheep and hog (Bonavolonta, 1956b), and confirmed for cattle (Sampaolo, 1952; Bonavolonta, 1956b). It is likely that the tendon endings in the other species mentioned, except for man, are of the palisade type.

14.32 Palisade endings.

Palisade endings were first described by Sherrington

(1897) in the eye muscles of cat and macaque monkey. He saw numerous nerve bundles pass into the tendon itself, subdivide, and either terminate there or else recurve back towards the muscle fibres to end at the musculo-tendinous junction. The terminal arborizations resemble those of a simplified tendon organ. In cat rectus and oblique muscles (Huber, 1900) a band of 25-30 palisade endings extends across the entire width of the tendons. According to Huber the palisade endings are derived from thinly-myelinated nerve fibres, enclosed in thin cylindrical capsules, and located mainly within the tendon. By comparison, the palisade endings illustrated by Dogiel (1906) in the eye muscles of the horse show a characteristic 'palisading' of nerve terminals around the ends of single muscle fibres. Complex palisade endings may in fact supply both ends of presumably short muscle fibres (see e.g. his fig.8) after the fashion of the basket motor endings found at both ends of muscle fibres in certain fish and amphibian muscles (see e.g. Bone, 1964; Barker, 1968). Dogiel (1906) also describes some comparatively rare and elaborate palisade endings supplied by thick nerve fibres that terminate on the ends of the thickest muscle fibres at the musculo-tendinous junction.

Dogiel's findings were largely confirmed by Tozer & Sherrington (1910) in cat, rabbit and monkey. Their illustration of the musculo-tendinous zone of monkey superior rectus muscle shows that most of the palisade endings are applied to the ends of the muscle fibres while a few are wholly within the tendon. Palisade endings have also been reported in the eye muscles of the camel (Crevatin, 1902) and are included in a schematic diagram of the innervation of

a human rectus eye muscle (Cooper et al.,1955) although not described in the text. A comparison of eye muscles from different classes of vertebrates (Sabussow, Maslow & Burnaschewa, 1964) has shown that palisade endings are first detectable in amphibia (frog species), are more complex in reptiles (lizard, snake and tortoise species), and most highly-developed in the eye muscles of mammals (cat and dog). They are absent from the eye muscles of birds such as pigeon, hen and owl.

The location of some palisade endings exclusively in tendon strengthens the view that they are 'sensorial' in nature as was first proposed by Sherrington (1897) and Tozer & Sherrington (1910). The possibility that palisade endings are motor in function has received no support from the work of Sas & Schab (1952). In the inferior oblique muscle of the cat, stimulation of a nerve branch that ends "solely" in palisade endings produced no visible contraction of the muscle fibres. However, since this nerve branch contains only thin muscle fibres it is possible that Sas & Schab were stimulating a grape-innervated 'slow' component in addition to palisade endings. Palisade endings may constitute either simplified tendon organs associated specifically with either single non-twitch muscle fibres or the muscle fibres of the peripheral layers (Barker, 1974), or alternatively they may represent particularly complex grape endings concentrated at the ends of the muscle fibres.

14.4 Endings in intramuscular connective tissue and tendon.

The intramuscular connective tissue of eye muscles may

contain Ruffini endings (Dogiel, 1906; Sas & Appeltauer, 1963), Dogiel endings (Dogiel, 1906), and a variety of encapsulated sensory nerve terminations (Wolter, 1955).

Golgi endings and Ruffini endings occasionally occur in the tendons of the eye muscles of man and other animals (Dogiel, 1906).

Very little information is available on the receptors present in the levator palpebrae and retractor bulbi group of eye muscles. Whereas the retractor bulbi and levator palpebrae of sheep are both relatively richly-supplied with muscle spindles (Cilimbaris, 1910), as are the rectus and oblique group, the retractor bulbi of cat contains no palisade endings (Huber, 1900).

SECTION B. RESULTS

Chapter 15. General morphology of muscle spindles in superior rectus and levator palpebrae.

The general morphology of sheep extraocular muscle spindles under the light microscope is similar to that of mammalian hindlimb muscle spindles (see Barker, 1974 for review). An intrafusal, or axial, bundle of 2-14 muscle fibres in SR (mean = 6.8) and 3-9 muscle fibres in LP (mean = 5.5) is enclosed over its middle portion by a fusiform capsule. This capsule expands in the equatorial region to form a fluid-filled periaxial space within which the axial bundle, enclosed by an axial sheath, is suspended from the capsule wall by strands of trabecular connective tissue. The capsule at this point is at its thickest and as it tapers towards the polar regions of the spindle it thins down and disappears.

15.1 The intrafusal muscle fibres.

Two types of intrafusal muscle fibre are present in the spindles of both SR and LP. There are usually one or two longer and thicker muscle fibres (mean = 18.8μm) that possess an accumulation of nuclei at the equator. These correspond to nuclear-bag fibres (Barker, 1948). The nucleation of such fibres is not, however, as prominent under the light microscope as that, say, of cat hindlimb nuclear-bag fibres. The remaining fibres are shorter, tapering off soon after leaving the capsule, and are thinner (mean = 11.2μm).

At the equator they contain a single central row of nuclei and are hence termed nuclear-chain fibres (Boyd, 1960). They frequently group together and pass through the spindle equator as a separate unit from the nuclear-bag fibres. Within such groups pairs of chain fibres are often closely associated and appear to fuse over parts of their length. That complete fusion does not occur can be ascertained only under higher resolution (see 18.123).

15.2 Compound and tandem spindles.

For such a small muscle, sheep SR is abundantly supplied with spindles that are further concentrated by being restricted to the relatively thin peripheral patch and orbital rim layers. Perhaps as a consequence of this they are often compound in form. Pairs of spindles may be arranged into parallel systems similar to those in rat tail muscles (Thompson, 1970). In sheep extraocular muscles the capsules of such parallel spindles often fuse to provide a common sheath that encloses both sets of intrafusal fibres (figs.187-188). Each set of intrafusal fibres may have a different equatorial region with an associated enlargement of the capsule. In addition to compound spindles, true tandem spindles occur, with a continuity of nuclear-bag fibres from one capsular region to the next, similar to those seen in the skeletal muscles of man (Cooper & Daniel, 1956), cat (Barker & Ip, 1961) and frog (Barker & Cope, 1962). These observations confirm those of Cilimbaris (1910), who described such spindles as 'multibellied' with up to five expanded capsule regions along the spindle length.

15.3 Incorporation of extrafusal muscle fibres.

In common with the parallel spindle systems in rat tail muscles (Thompson, 1970) and the spindles in the lumbrical muscles of man (Cooper & Daniel, 1963) it is not unusual for the spindles in sheep SR to be associated with extrafusal fibres. These may either be incorporated in a separate compartment by the outer layers of the capsule (see fig.218), or may completely penetrate the periaxial space. Cilimbaris (1910) described the fibres that enter or leave the spindle via the capsule as Zwischenpolfasern. In the present study such fibres were usually briefly encapsulated and did not receive any sensory innervation. More rarely, 'extrafusal' fibres become fully incorporated with the axial bundle and receive a similar sensory supply. It has already been mentioned that some of the nuclear-bag fibres at the insertion end of SR consist of daughter fibres derived from the division of peripheral grape-innervated muscle fibres (see chapter 11).

Chapter 16. Number, distribution, and length of spindle capsules in superior rectus and levator palpebrae.

The single SR and LP muscles examined in serial transverse section contained 181 and 61 spindle capsules, respectively. Camera lucida drawings of the muscles were made every twenty sections (200μm) to provide a basis for accurate mapping and tracing of the spindle capsules, and sections were examined in between these drawings. Measurements and counts were restricted to spindle capsules since individual tracing of long intrafusal fibres would have been extremely difficult. The figures compare with the counts of 148 for SR and 23 for LP made by Cilimbaris (1910). He examined serial 50μm sections of the whole orbital content, and may thus have missed some of the very short capsules.

The spindles are fairly evenly distributed throughout the lengths of both muscles, as may be seen from the projection plan of the muscles (fig.189) in which the positions and lengths of the capsules are plotted within the limits of a single representative longitudinal section. Up to 26 spindle capsules were present in a single transverse section through SR. These spindles were located mainly around the dorsal and lateral periphery, in either the orbital rim or peripheral patch layers and are absent from the muscle portions adjacent to the eyeball (fig.190). In the proximal third of SR, 64 of the 70 spindles present were located either within the peripheral patch layer at the extreme end of the muscle, where there is no orbital rim layer; or towards the belly, in the junctional region between the peripheral patch and orbital rim layers. Six

spindles were located in the peripheral fascicles of the central core.

The belly third of the muscle lacks a peripheral patch layer, and the 70 spindles were located almost exclusively within the orbital rim layer; only two spindles were found in the central core. The insertion third of the muscle contained 41 spindle capsules, of which 8 were located in the peripheral fascicles of the central core. The remainder were located either in the junctional region between the peripheral patch and orbital rim layers, where the orbital rim is present; or within the peripheral patch layer at the distal end of the muscle, where the orbital rim layer is absent.

In LP the maximum number of spindle capsules in any transverse section was 14 and their distribution within a transverse section is random (fig.144). In both SR and LP the extra- and intrafusal muscle fibres are orientated parallel to the long axis of the muscle.

The mean capsule length of the 181 spindles in SR was 3.8mm within a range of 0.04-14.5mm. The 61 spindles in LP had a mean capsule length of 3.7mm within a range of 0.04-13.3mm. Since, in cat hindlimb spindles, the length of the capsule varies according to the number of sensory endings present, the relatively long capsules in sheep extraocular muscle spindles are indicative of a profuse supply of sensory endings.

Chapter 17. Histochemistry of intrafusal fibres.

17.1 Extraocular muscle spindles.

The muscle-spindle histochemistry of both SR and LP is similar. Individual nuclear-bag and nuclear-chain fibres were identified at the equator with W & VG stain, and then traced to the juxta-equatorial region to demonstrate histochemical activity.

The majority of nuclear-bag fibres exhibit a type G histochemical profile comparable to that shown by the peripheral G fibres in SR (figs.191-211). The level of activity for P'ase, SDH and Alk ATPase is thus relatively low, whereas with Acid ATPase a high activity is given. The colouration with P'ase appears fawn, or light brown, and a 'pale' pattern of numerous small formazan particles is seen with SDH. Alk ATPase activity is minimal with a white reaction product, whereas an intense black colouration is given with Acid ATPase.

In SR 84 (85.7%) of 98 bag fibres from 58 spindles conformed to the above pattern. In the remainder (14.3%) the activity with either P'ase or Alk ATPase was slightly higher, but not significantly so (fig.195). SDH activity in these fibres was always low and for Acid ATPase was always intensely high. Although it is possible that these few bag fibres represent an intermediate type of bag fibre, as has recently been distinguished in mammalian skeletal muscle spindles (see e.g. Barker, 1974 for a review), it seems more likely that they are simply a product of normal minor variations in the staining of extraocular bag fibres.

The nuclear-chain fibres of both SR and LP (figs.191-211) exhibit a type C histochemical profile comparable to that of the small C fibres of SR. High activity is given with P'ase (blue-black colouration), SDH (a 'red' pattern of uniformly-distributed large formazan particles) and Alk ATPase (black reaction product). Acid ATPase activity is low with some faint residual staining.

With any given stain the level of activity remains constant throughout the juxta-equatorial and polar portions of the spindle. In the case of Acid ATPase this feature is particularly important, since a recent report by Yellin (1974) on rat spindles, and a demonstration by Harriman, Elliot & Parker (1974) on human spindles, have both indicated that one type of bag fibre may reverse its Acid ATPase staining characteristics as it passes from the equatorial to the polar regions of the spindle. This particular type of bag fibre is the one that normally exhibits low Alk ATPase activity throughout its length. That the bag fibres in sheep SR and LP muscles do not show such regional variation in their Acid ATPase activity is another indication that the intermediate type of bag fibre is not present in extraocular muscle spindles.

The nucleated equatorial regions of the intrafusal muscle fibres showed reduced staining characteristics for all histochemical stains, and the reaction products tend to be restricted to a rim around the periphery of individual fibres (fig.188).

Within the peripheral patch layer at the insertion end of

SR isolated observations have been made of spindles that contain only one or two intrafusal fibres. The single encapsulated fibres have identical staining properties to the surrounding intermediate G fibres and are also comparable in diameter. They appear, to all intents and purposes, as encapsulated 'extrafusal' G fibres. Since it was not ascertained in serial transverse section that such fibres possess an equatorial nucleation with presumed sensory innervation, it cannot be stated that they constitute true monofibral spindles that would consist of one nuclear-bag fibre only.

The spindles that contained two fibres were composed of a single bag and a single chain fibre (fig.197 series). In other respects they were not exceptional.

17.2 Peroneus brevis spindles.

In the sheep hindfoot PB muscle (figs.212-217) the staining characteristics of the chain fibres correspond to those of the chain fibres in SR and LP spindles. They show high activity for P'ase, SDH and Alk ATPase. There are, however, clearly two types of bag fibre both of which exhibit different histochemical profiles to the bag fibres in SR and LP.

The slightly shorter bag fibre in PB, although much thicker than the nuclear-chain fibres, possesses a similar histochemical profile in terms of P'ase, SDH and Alk ATPase activity (figs.212-214). In some respects therefore it might be regarded as an enlarged chain fibre with a greater accumulation of nuclei at the equator.

The longest bag fibre also shows a high activity for SDH (fig.213). In fact, the intrafusal fibres cannot be clearly distinguished with SDH; they all show similar intensely-active profiles that are much darker than the surrounding extrafusal fibres. The longest bag fibre exhibits relatively low to minimal (figs.212 & 215) Alk ATPase activity, and moderate to high (figs.214 & 217) P'ase activity. In some spindles in PB the type of bag fibre with low Alk ATPase activity is absent, and the bag and chain fibres are distinguishable only by diameter and equatorial nucleation.

The type of histochemical profile exhibited by both the chain fibres and the shorter bag fibre corresponds to that exhibited by the extrafusal conventional C type that constitutes 39% of the total number of extrafusal fibres. The longest bag fibre has a modified type C histochemical profile comparable to that present in 60% of the surrounding extrafusal fibres. Whereas all intrafusal fibres have a capacity for combined oxidative and glycolytic metabolism, the chain fibres and shorter bag are presumably fast-contracting and the longer bag is probably slow-contracting.

Chapter 18. Ultrastructure of muscle spindles.

18.1 Extraocular muscles.

18.11 Capsule and axial sheath.

At its thickest part the capsule consists of 5-8 concentric layers of thin, flattened 'capsular sheet cells' (Merrillees, 1960), each of which closely interdigitates with its neighbour so that an almost continuous cell layer is formed (see fig.218). The cell layers alternate with layers of collagen fibrils and occasional elastic fibrils that are usually longitudinally orientated.

The cytoplasm of the capsular sheet cells contains small mitochondria, polyribosomes and glycogen granules, and both sides of the cell membrane are lined by numerous pinocytotic vesicles.

Basement membrane is usually present on both sides of capsular sheet cells, but in cells that pair to form channels, or zones of close apposition, basement membrane is absent.

The innermost layer of the capsule is composed of fibrocyte cells (fig.218) some of which traverse the periaxial space to join similar cells that form the axial sheath. These cells resemble capsular sheet cells but are more electron dense, contain fewer pinocytotic vesicles, and lack basement membrane. Fibrocyte cells have occasionally been observed in the outer layers of the spindle

capsule.

In the equatorial region of the axial bundle the fibrocyte cells are numerous and almost completely surround individual intrafusal fibres (e.g. fig.227). In the polar regions of the spindle several intrafusal fibres may be grouped within a single fibrocyte cell. Associated with the fibrocyte cells are collagen fibrils, elastic fibrils and non-myelinated pre-terminal axons. Blood capillaries have been observed between the capsule lamellae (fig.218), but not within the periaxial space.

18.12 Intrafusal muscle fibres.

In SR and LP muscles the ultrastructure of the bag and chain fibres is comparable to that of the peripheral G fibres and the small C fibres, respectively in SR.

18.121 Nuclear-chain fibres.

A transverse section through the polar region of a chain fibre (fig.220) shows the myofibrils to be small and discrete at the I band level where they are encircled by a single layer of beaded tubules of sarcoplasmic reticulum. The large mitochondria have tightly-packed cristae and are scattered evenly throughout the muscle fibre. Glycogen granules are abundant and are commonly associated with the profuse sarcoplasmic reticulum at the I band level, but may also be distributed within the myofibrils. Micropinocytotic vesicles are often present beneath the sarcolemma.

In longitudinal section the chain fibres of SR (figs. 221-222) and LP (fig.226) show distinct M lines in the middle of pseudo-H zones, and relatively thick, wavy Z lines that exhibit Z filaments. The mitochondria are elongate and form intermyofibrillary chains that often include lipid droplets. Triads are regular and frequent at the junction of the A and I bands with usually one and often two triads per sarcomere. They may occasionally be longitudinally orientated (fig.220). The sarcoplasmic reticulum and associated glycogen granules are most extensively developed in the I band.

In the polar regions nuclei are usually peripheral, but in the myotube and equatorial regions they become centrally placed to form a chain. A transverse section through the equatorial region of a chain fibre (fig.227) shows one nucleus, or occasionally a pair of overlapping elongate nuclei, surrounded by a thin peripheral band of myofibrils. The nuclei at the ends of the chain are less tightly-packed than those at the equator and are separated by sarcoplasm rich in glycogen, ribosomes and aggregations of mitochondria.

18.122 Nuclear-bag fibres.

In their polar regions the bag fibres are notable for their poorly-delineated and irregular myofibrils. A transverse section through the I band (fig.219) shows only irregular beaded tubules of sarcoplasmic reticulum encircling the large myofibrils, whereas in the A band there is no delineation at all. The mitochondria, which are scattered evenly throughout the muscle fibre, are smaller,

fewer and the cristae less tightly-packed than those of the chain fibres. Triads are comparatively rare and the tubules are occasionally longitudinally orientated. The sarcoplasmic reticulum, which is only poorly-developed at the I band level, is associated with glycogen granules that are generally fewer in number than in chain fibres. Occasional patches of densely concentrated glycogen granules have been observed in longitudinal section (fig.223). As in chain fibres, micropinocytotic vesicles are commonly seen beneath the sarcolemma.

In longitudinal section through the bag fibres of SR (figs.223-224) and LP (fig.225) an M line is absent from the middle of an ill-defined and wavy pseudo-H zone, and the Z lines are relatively thick, with Z filaments, and also exhibit a wavy appearance. The mitochondria are elongate and may extend across a sarcomere, but do not usually form chains. Triads occur at the junction of the A and I bands, but are infrequent and irregular in occurence.

In the myotube region the nuclei lie centrally and are surrounded by a thin peripheral rim of myofibrils in a similar fashion to the chain fibres. At the level of the nuclear bag, however, there is an increase in the number of nuclei so that a maximum of five nuclei may lie abreast within the muscle fibre (fig.228).

18.123 Close apposition of intrafusal fibres.

As intrafusal fibres were traced in transverse section through the intracapsular region several instances of close apposition of pairs were observed. This was a comparatively common occurence between chain fibres (figs.229-230), which are usually closely grouped in this region, but a single instance of apposition of two bag fibres (figs.231-234) was also seen.

Over such regions of apposition in chain fibres, two associating fibres come together and are enclosed by a common basement membrane. The two paired fibres are seen as a single oval contour in transverse sections at low magnification and they approximate to the appearance of the apparently-fused chain fibres seen under the light microscope. At high magnification, however, no instances of real fusion were seen. Where the plasma membranes of the paired fibres are so closely apposed that the basement membrane is absent from their common border, the fibres remain separated by a gap of about 20nm.

The close apposition of chain fibres and their intertwining within the axial bundle may partially explain the light-microscope observation (Cilimbaris, 1910) of splitting and anastomosing intrafusal fibres in sheep extraocular muscle spindles. Comparable regions of close apposition between chain fibres have been observed in cat muscle spindles (Corvaja, Marinozzi & Pompeiano, 1967; Adal, 1969).

In the single case of close apposition between bag fibres the initial contact was in the form of a pseudopodial projection from one bag to the other (figs.232-233). At the base of the projection the plasma membranes of the two fibres were separated by about 20nm and the basement membrane was reflected between the fibres. The region of close apposition was gradually extended (fig.234) until the two bag fibres were linked over about one tenth of their perimeters.

18.2 Peroneus brevis.

18.21 Intrafusal muscle fibres.

In the juxta-equatorial (fig.236), polar and extreme polar regions of spindles from PB it is possible to distinguish two broad categories of intrafusal fibre of large and small diameter. At the equator (fig.235) there is a marked reduction in diameter of all intrafusal fibres and the difference in diameter between large and small fibres is not so apparent.

The fibres of small diameter are always of the nuclear-chain type. They frequently group together as they pass through the equatorial region. They exhibit similar ultrastructural characteristics to the chain fibres in SR and LP spindles. Thus they possess an M line and have well-delineated myofibrils, large mitochondria, and abundant sarcoplasmic reticulum and glycogen granules (figs. 239 & 242). Dilated cisternae of the sarcoplasmic reticulum have also

been observed apposed to mitochondria.

The fibres of large diameter possess a nuclear bag, but do not form a homogeneous group. They are of two types, which for convenience are termed typical bag and intermediate bag (Barker, 1974), respectively. The typical bag fibre is the longest, and although it contains a higher number of mitochondria than the bag fibres of SR and LP it nevertheless also shows some comparable ultrastructural features. In transverse section the myofibrils are irregularly-delineated in the I band by poorly-developed sarcoplasmic reticulum, and hardly delineated at all in the A band (fig.237). In longitudinal section (fig.240) triads or dyads are irregular and infrequent in occurence at the junction of the A and I bands; the sarcoplasmic reticulum and associated glycogen is only poorly-developed in the I band; and the mitochondria are short and do not form chains. However, in contrast to the bag fibres in SR and LP, the Z line is comparatively straight and relatively thin; and although a prominent M line is absent, two faint parallel lines separated by a lighter interspace are present within the pseudo-H zone. Similarly-structured 'double' M lines have been observed in the typical bag fibres of cat (Barker, 1974) and rat (Ovalle, 1971; 1972) lumbrical muscles.

The intermediate bag fibre in PB is slightly shorter than the typical bag fibre and usually has a smaller nuclear bag. Compared to the typical bag fibre, the intermediate type (figs.238 & 241) has larger mitochondria that are longitudinally-elongated and

form intermyofibrillary chains; more abundant sarcoplasmic reticulum and glycogen; a thicker Z line; more frequent triads or dyads at the junction of the A and I bands; and a prominent M line in the middle of the pseudo-H zone. In its ultrastructure the intermediate type thus exhibits some characteristics (e.g. M line, large mitochondria) common to chain fibres and also some characteristics (large diameter, nuclear bag) associated with typical bag fibres. Although a direct ultrastructural/histochemical correlation of intrafusal fibre types has not been attempted, it is presumed that the typical bag fibre, because of its greater length, corresponds to the bag fibre with a 'modified' C-type histochemical profile. The intermediate bag thus corresponds to the bag fibre exhibiting a conventional C-type histochemical profile.

Chapter 19. The sensory innervation.

19.1 Light microscopy of extraocular muscle spindles.

With the silver-impregnation methods used, the thick, myelinated sensory afferents that supply sheep extraocular muscle spindles were well-stained up to their final subdivision near to the intrafusal muscle fibres. The terminations themselves, however, were not generally as intensely stained as those of the motor nerves within the same spindle. Where the terminations are distinct (figs.243-248) they may be distinguished into forms that correspond, in general appearance, to either the primary or the secondary sensory endings found in cat hindlimb spindles (see Barker, 1974). This confirms the previous observations of primary and secondary endings in sheep extraocular muscle spindles by Cilimbaris (1910) and Denny-Brown (1928).

19.11 The primary ending.

Each spindle receives a single, thickly-myelinated primary axon. In SR the diameter of 41 primary afferents, including the myelin sheath, ranged from 7.94μm to 14.69μm with a mean of 11.1μm. In LP 20 primary axons had diameters of 6.5-14.1μm with a mean of 9.9μm. Measurements were made about 1mm from spindle entry. In SR the slightly greater mean diameter of primary afferents is due in part to a greater number of spindles in which secondary sensory endings are also present and in which the primary afferents tend to

be thicker (see also Adal & Barker, 1962).

The primary afferent in extraocular muscle spindles usually enters the capsule obliquely and divides dichotomously (fig.243). Nearer the intrafusal fibres the two main branches sub-divide to give several terminal branches that supply the nucleated regions of both bag and chain fibres. The axon terminals take the form of half rings and annulospirals that are comparatively compactly arranged. The tight coils around respective intrafusal muscle fibres give a good indication of their diameter, and where both bag and chain fibres are innervated thick and thin coils are evident (figs.244-245).

19.12 The secondary ending.

Individual spindle capsules may have up to 4 secondary endings, derived from slightly thinner myelinated axons than the primary endings and usually located on one side of them. In SR the diameter of 40 secondary afferents ranged from 5.9μm to 9.4μm, with a mean of 7.52μm. In LP 14 secondary axons had diameters of 4.9-8.8μm with a mean of 7.0μm. In both SR and LP there is some overlap in diameter between the thinner primary and thicker secondary afferents (fig.249). In SR, of 10 spindles supplied with a primary afferent only, 8 were supplied by primaries of less than 10μm in diameter. These constitute only half of the total primary afferents in the overlap range, so the overlap cannot be totally attributed to the small diameter of the primaries that supply simple spindles (primary endings only). In LP, however, 6 of the 8

primary afferents in the overlap range supplied simple spindles.

The secondary afferents in SR and LP usually enter the spindle capsule parallel to the intrafusal muscle fibres and are usually separate from the primary afferent (fig.246). The axon terminals of the secondary ending are distributed mainly to the chain fibres (figs.247-248) and are generally more dispersed than those of the primary ending and extend over a greater length. They are composed of fine tendrils that encircle the intrafusal fibres to form an irregular annulospiral network with occasional knob-like synaptic contacts.

19.13 The pattern of innervation.

In SR, of 41 capsular regions sampled from single, tandem and parallel spindles, 10 (24.4%) received a primary ending only (P-only combination) and 25 (61.0%) received a primary and a single secondary supply (PS combination). The remaining 6 spindles (14.6%) were complex and received a primary and more than one secondary ending: 4 had two secondary endings (3 PSS combinations & 1 SPS combination); 1 had three (PSSS combination); and 1 had four (PSSSS combination). The most usual condition in spindles from SR is therefore for there to be a single secondary ending lying adjacent to a primary ending, and in fact the total number of primary and secondary endings in the spindles sampled was approximately equal. There is a similar overall ratio of secondary to primary endings in cat hindlimb spindles (see e.g. Barker, 1962; Boyd, 1962).

As far as could be determined from teased preparations, no branching of the primary and secondary endings occurs to supply adjacent spindles. If, as in cat, an approximately 1:1 ratio is maintained between primary or secondary afferents and muscle spindles, then the 150-180 spindle capsules in SR would receive a total of approximately 300-360 large-diameter afferents.

In LP, of 20 capsular regions analysed for mode of sensory innervation, 9 (45%) received a primary ending only, 8 (40%) had a PS combination, 2 (10%) had a PSS combination, and 1 (5%) had a SPS combination. Compared to SR, a greater proportion of spindles in LP appear innervated by primaries only, relative to the PS combination. The secondary sensory innervation is also reduced, the ratio of primary to secondary endings being 1:0.7. The 60 or so capsular regions in LP might be expected to receive a total of about 100 primary and secondary afferents.

19.2 Light microscopy of peroneus brevis spindles.

In 33 spindles from PB the diameters of the primary afferents ranged from 6.8-13.2µm with a mean of 9.48µm. In contrast to the extraocular muscle spindles the primary afferents normally enter the capsule at right angles to the axis of the spindle, and are frequently associated with any entering secondary afferents. The 17 secondary axons had diameters of 5.3-8.2µm (mean = 7.26) and thus overlapped the lower end of the primary diameter range (fig.249). Of 18 primary afferents supplying P-only spindles, 12 have diameters less than

10μm. However, these constitute only half of the primary afferents in the overlap range, as in SR.

The mode of sensory innervation of the 33 spindles in PB selected for analysis was as follows: 18 (54.5%) received a primary ending only; 13 (39.4%) had a PS combination; 1 (3.0%) had a PSS combination; and 1 (3.0%) had an SPS combination. Compared to LP there is an even greater proportion of spindles with primaries only, and an even further reduction in the amount of secondary innervation; the ratio of primary to secondary endings being 1:0.52.

Figure 249 shows the distribution of primary and secondary sensory nerve-fibre diameters (including the myelin sheath) for SR, LP and PB muscle spindles.

19.3 Electron microscopy.

The sensory terminals in the muscle spindles of PB, SR (figs.250-254) and other sheep extraocular muscles (Scalzi & Price, 1972) have a similar ultrastructure to those described in other species (see Barker, 1974). It was not possible to distinguish the axon terminals of primary and secondary endings on the basis of difference in shape as has been proposed for cat (Corvaja, Marinozzi & Pompeiano, 1969), and rat (Mayr, 1970) terminals. The sensory terminals on both bag and chain fibres usually have a low and flattened ellipsoid profile and do not bulge outward from the muscle fibre in transverse section. However, near to and at the equator,

sensory terminals are commonly more extensive and may in some cases almost encircle the intrafusal fibres in a crescentic fashion (fig.251). These forms presumably represent the annulospirals of the primary endings that supply both bag and chain fibres. In the juxta-equatorial region the less extensive axon terminals that are located on chain fibres are probably secondary sensory endings.

The sensory axon terminals contain numerous mitochondria; frequent clear vesicles and occasional granular vesicles; multivesicular bodies containing from 3-6 vesicles; occasional neurofilaments; and glycogen granules. They lie in shallow troughs on the circumference of the intrafusal muscle fibres and are enclosed by a common basement membrane. At the margins of the trough the sarcolemma forms thin lips that extend over the outer surface of the ending for a variable distance (fig.251). At times the sensory ending may be completely enclosed by sarcoplasm (fig.250). The sensory myoneural junction is usually smooth with no junctional folds, but occasional pseudopodial projections may interdigitate with the underlying myofibrillar material (fig.254).

On one occasion an unusual structure with some resemblances to a sensory terminal was observed (fig.253). This was located on a chain fibre in the myotube region of a spindle in SR. It was about twice the size of a sensory terminal and embedded in a similar trough in the muscle fibre, but was densely packed with mitochondria. A similar expanded 'sensory-like' axon terminal has been observed in tortoise muscle spindles (Crowe & Ragab, 1970).

In many cases sensory terminals make simultaneous synaptic contact with two or, more rarely, three neighbouring muscle fibres. Such "sensory cross-terminals" (Adal, 1969) usually occur between chain fibres that may be either paired (fig.250) or in close apposition (fig.230). This pattern of cross sensory innervation between chain fibres in sheep extraocular muscle has also been noted by Scalzi & Price (1972). In addition, one instance in SR of a sensory cross-terminal between a bag and a chain fibre (fig. 252) was observed.

Chapter 20. The fusimotor innervation.

20.1 Extraocular muscle spindles.

20.11 Light microscopy.

The motor innervation of 94 muscle spindles was studied in teased, silver preparations of whole spindles, of which 64 were from SR and 30 from LP. In both SR and LP spindles two types of motor ending, plate and grape, are found that have a similar appearance to their extrafusal counterparts, except that they tend to be smaller.

The discrete end-plates are located mainly in the juxta-equatorial regions of the spindles in SR (figs.255-256) and LP (figs.260-262) on either side of the sensory endings. They are derived from one or two myelinated axons of diameter 2.8-5.9μm (mean = 4.09μm) that often enter the capsule with the primary or secondary sensory supply, pass out along the intrafusal muscle fibres toward the poles, and branch to give 4-6 end-plates that terminate on chain fibres. The end-plates frequently lie at the same level on adjacent chain fibres to form a small 'terminal innervation band'.

Other plate endings occur singly in the mid-polar regions of the spindle (figs.257-259) and are usually supplied by separate axons that enter the spindle at the poles near to their terminations. These axons often pass to the spindle from small nerve bundles that terminate in extrafusal end-plates. Occasionally a clear collateral

derivation of intrafusal end-plates from a nerve-fibre supplying extrafusal plate endings can be seen. For clarity, the intrafusal plate ending shown in fig.257 has been traced at lower magnification with its extrafusal collateral counterpart (fig.271a).

The multiterminal grape innervation in SR (figs.263-267) and LP (figs.268-270) is more diffuse than the plate innervation and is located mainly in the polar and mid-polar regions of the spindle on the bag fibres. The grape endings are derived from myelinated axons that range in diameter from 1.2-4.8μm (mean = 2.96μm). These parent grape axons often enter the spindle at the poles and pass along the bag fibres, giving off at intervals short, non-myelinated terminal branches from the nodes that terminate in a series of knob-like synaptic contacts. These are usually linearly arranged (see e.g. figs.265-269), but may also provide a classic 'bunch of grapes' appearance (fig.270). The innervation zones so formed are shorter than those of the extrafusal peripheral G fibres in SR.

In SR the common origin of intrafusal and extrafusal grape endings is frequently apparent in the polar regions of the spindle. A tracing of a typical collateral grape configuration (fig.271b) may be compared to a similar illustration given by Cilimbaris (1910; figure 10). Grape axons have also been observed to leave a spindle after supplying some grape innervation to bag fibres and finally terminate either on extrafusal muscle fibres or within a second spindle.

In LP the grape innervation of bag fibres is also easy to demonstrate in the polar regions of the spindle, but the innervation is sparser than in SR with the innervation zones more widely spaced. The grape endings are not collaterally derived, since there is no extrafusal grape innervation. In every case where an apparently grape-innervated extrafusal muscle fibre was teased it proved to be the polar portion of a bag fibre. Thus in LP the grape supply is specifically fusimotor.

20.12 Electron microscopy.

The ultrastructure of the intrafusal plates on the chain fibres in SR (fig.272-274) and LP (fig.275) shows compactly-arranged axon terminals that lie in shallow depressions of a thinly-spread sole plate, with regular and frequent post-junctional folds. The junctional folds are unbranched, short, wide and expanded at their bases and the basement membrane lines the sides of the folds to give a 'vacuolar' appearance. The axon terminals contain the usual organelles including mitochondria, clear synaptic vesicles, and occasional dense-core vesicles (fig.274). In figure 272 a longitudinal section through the juxta-equatorial region of a spindle in SR passes through the raised sole-plate region of a plate ending before the underlying chain fibre has been reached. The compact nature of the plate endings is clearly seen with six axon terminals clustered around a central sole plate. The terminals are derived from a myelinated axon of about 3.0μm in diameter.

In SR the ultrastructure of the intrafusal plates closely resembles that of the plate endings on the extrafusal small C muscle fibres that surround the spindles in the peripheral patch and orbital rim layers.

The grape axon terminals on the bag fibres in SR (figs. 277-279) and LP (fig.276) correspond in their ultrastructure to the axon terminals on the extrafusal G fibres in SR. They are usually applied to the surface contour of the muscle fibre with no depressions or guttering. The sole plate, if present, is very thinly spread, and the myoneural junction is mainly smooth. Occasional, short junctional folds may be present that have expanded bases. These appear either 'flask-shaped' or 'vacuolar' in form, and are lined by basement membrane. The grape terminals in longitudinal section (fig.277) extend for greater distances along the muscle fibre than the terminals of the plate endings and appear more linear in their arrangement. They contain the usual organelles including dense-core vesicles. Short microladders or 'leptofibrils' have been occasionally observed in the sarcoplasm underlying the grape axon terminals.

A schematic representation of the pattern of motor innervation in the spindles of SR and LP is given in fig.280. The collateral plate innervation in SR is presumed to be derived from axons innervating small C fibres that surround the spindles in the peripheral patch and orbital rim layers, and which possess identical ultrastructural and histochemical properties to the chain fibres. In LP the derivation of the fusimotor plate collateral from the C-type fibres rather than from the B-type fibres is conjectural.

20.2 Peroneus brevis spindles.

20.21 Light microscopy.

The fusimotor innervation in sheep PB muscle was studied in 45 teased, silver preparations of whole spindles. These were found to contain similar types of motor ending to those present in the cat hindlimb muscle spindle, i.e. p1 and p2 plates and trail endings (see Barker, 1968; 1974).

Trail endings. The most profuse type of ending in PB resembles the trail ending in cat hindlimb spindles in that it is mainly juxta-equatorial in location and is derived from extensively branched axons of small diameter (figs.282-283). The trail axons characteristically enter the capsule with the sensory innervation and often branch so as to innervate both poles. In each pole the axons branch repeatedly and terminate in simple twig-like endings that may be distributed to bag fibres only (fig.282), chain fibres only, or, most commonly, to both bag and chain fibres (fig.282). One instance was observed where the total motor supply to a spindle consisted of a single trail axon that ramified throughout the two poles to give a total of 19 simple endings on bag and chain fibres. Although the term 'trail' does not appropriately describe the form of these endings in PB they are presumed to be homologous with the trail endings in cat that are supplied by specifically fusimotor axons.

p2 plates. In the polar and mid-polar regions of the spindle there are large, compact plate endings (figs.281 & 284) which, in contrast to the p1 plates, have no obvious sole plate, are generally larger and more elongate, and have more numerous axon terminals in the form of knobs and rings. They are supplied by axons of relatively large diameter that usually enter the spindle capsule with the sensory supply and pass out toward the poles where they branch and terminate on both bag and chain fibres. It is presumed that, as in cat, the p2 plates are derived from specifically fusimotor γ axons.

p1 plates. In the polar regions of the spindle there are occasional single plate endings (figs.285-286) that in general appearance closely resemble the motor end-plates on extrafusal muscle fibres (figs.287-288). These endings are mainly confined to the bag fibres and are usually supplied by axons that leave neighbouring intramuscular nerve trunks and enter at the spindle pole. Although the collateral derivation of the p1 plates from axons supplying extrafusal end-plates has not been established in the present study, it is presumed that in PB the p1 plates are supplied by skeleto-fusimotor β axons, as in cat hindlimb spindles.

20.22 Electron microscopy.

In PB several spindles were sectioned transversely through to the mid-polar or juxta-equatorial regions and then turned through 90^{o} and sectioned longitudinally. In the cat the ultrastructure of the p1 plate (Barker, Stacey & Adal, 1970; Goglia, 1970) closely

resembles that of extrafusal end-plates, with the axon terminals lying in synaptic gutters on a substantial sole plate, and with long, narrow and frequent junctional folds. It was not possible in the several spindles analysed from PB to identify a similarly structured pl plate ending under the EM. This failure is presumably related to the small size of the pl plates and their relatively low frequency.

In the mid-polar and juxta-equatorial regions of the spindle, however, the typical bag (fig.289), intermediate bag and chain fibres (fig.291) receive a profuse innervation. In addition to numerous axon terminals that are scattered along the length of the intrafusal fibres, there are also many non-myelinated pre-terminal axons that lie above the surface of the muscle fibres. Some of the pre-terminal axon bulbs are connected by strands of axoplasm to the terminal axon bulbs (fig.291) which may themselves be interconnected. The shape of the axon terminals is thus irregular and they are generally larger than those of the intrafusal grape terminals in SR and LP. In addition to the organelles seen in the terminals of SR and LP there are also numerous densely-staining structures that appear to be multivesicular bodies packed with vesicles. The dense-core vesicles also appear more abundant than in SR or LP.

In the juxta-equatorial regions of the spindle the terminals are applied to the surface of the intrafusal muscle fibres with either little or no underlying sole plate and no regular folding of the post-junctional membrane (figs.289-291). The occasional folds are short

and bulbous. These terminals probably correspond to the trail endings seen in teased, silver preparations, and although the terminals are in the form of knob-like synaptic contacts rather than extended along the muscle fibre as in cat trail terminals, the form of the myo-neural junction is similar. They occur on both bag and chain fibres.

In the mid-polar regions of the spindle, endings have been observed on intermediate bag (figs.292-293) and chain fibres that are composed of more compactly arranged axon terminals that lie over a thinly-spread sole plate. Whereas the post-synaptic membrane remains smooth in some regions, in others it is thrown into regular, short bulbous folds. Such endings may possibly correspond to the p2 plate endings seen in teased, silver preparations, although the folding is not as extensive as that seen beneath p2 plates in cat (Barker, Stacey & Adal, 1970), and the folds are not confluent at their bases.

Chapter 21. Other receptors in sheep extraocular muscle.

The present study has confirmed the observations of Bonavolonta (1956b) that tendon organs are present in the rectus and oblique extraocular muscles of sheep. Although extensive teasing was carried out at the insertion end of SR, tendon organs were encountered only occasionally and it is probable that the total number in each muscle is of the order of only 4-6, as in the ox (Marchi, 1882). The several tendon organs that were isolated were all conventional in their morphology (figs.294-295). Each consisted of a thin fusiform capsule enclosing a bundle of tendon fascicles that received numerous terminal arborizations from a large-diameter afferent. The tendon organs were all located at the musculo-tendinous junction and were attached to 3-13 muscle fibres, usually at the proximal end, but occasionally half way along the capsule (fig.295).

The muscle fibres attached to the tendon organs were either all plate-innervated and of generally large diameter, probably from the central core layer, or, in the peripheral patch layer consisted of both plate- and grape-innervated fibre types that were associated with the muscle spindles. The tendon organs do not therefore appear to be restricted to a specific layer of the muscle or to a particular type of muscle fibre.

It should be noted that all of the species in which typical tendon organs have been observed, (man, ox, hog, sheep and horse;

see 14.31) also possess muscle spindles (see 14.1).

In sheep SR, palisade endings (14.32) are absent, and simple spiral endings on extrafusal muscle fibres have not been observed.

SECTION C. DISCUSSION

The presence or absence of muscle spindles in the extraocular muscles of various species does not appear to be closely related to phylogeny. Whereas spindles are present in the extraocular muscles of a wide range of artidactyls, the horse, and man, they are absent from the extraocular muscles of all the carnivores so far examined, most of the rodents (except mouse), and also from certain of the monkeys (14.1). No information is available as to the occurrence of muscle spindles in the extraocular muscles of monotremes, marsupials, or other placental mammals, including the aquatic species.

The absence of spindles from extraocular muscles may, however, be less significant than at first appears. Several species, including rabbit, cat, rhesus monkey and mangabey monkey, that have no conventional muscle spindles in their eye muscles (14.1), nevertheless do have simple spiral endings in contact with otherwise unspecialized extrafusal muscle fibres (14.2). Some spirals, at least, supply muscle fibres of small diameter in the peripheral layers of the muscle (see e.g. Cooper & Fillenz, 1955; Sas & Appeltauer, 1963) where spindles would normally be located. These endings probably correspond to the muscle stretch receptors identified in the mangabey monkey and the cat (Cooper & Fillenz, 1955; Bach-y-Rita & Ito, 1966b) that are distinct from muscle spindles and tendon organs. It thus appears that in those species that lack spindles in

their eye muscles the role of the muscle stretch receptor is performed by the spiral endings. In man, horse, cow and albino mouse, the extraocular muscles contain both spiral endings (14.2) and muscle spindles (14.1), both of which presumably operate as stretch receptors in these animals. In the superior rectus muscle of the sheep, where spindles are extremely numerous, spiral endings are apparently lacking. The inferior oblique and lateral rectus muscles of the squirrel monkey (Ito & Bach-y-Rita, 1969) are exceptional in that they not only lack spindles, but there is no evidence for any other type of muscle stretch receptor.

The question remains as to why certain species should possess conventional spindles in their eye muscles while other species do not. Cooper & Daniel (1949) have made the point that in many animals "neck movements largely take the place of extensive eye movements" whereas others "have no foveae and do not depend, to any major extent, on eyesight". The extraocular muscles of such animals not only lack spindles, but they also receive a less profuse nerve supply.

Where spindles are present in extraocular muscles a notable feature is their almost exclusive location within the peripheral layers of the rectus and oblique muscles. This is presumably related to their pattern of motor innervation. In sheep SR the spindles receive a collateral innervation from axons that supply either the plate-innervated small C fibres or the grape-innervated small-intermediate G fibres that are concentrated in the orbital

rim and peripheral patch layers. A similar situation occurs in amphibian layered muscles, such as iliofibularis, in which the spindles are found among or close to the aggregation of plate- and grape-innervated fibre types from which a collateral innervation is derived (see e.g. Smith et al.,1973; Barker, 1974).

With reference to individual species, the general morphology of extraocular muscle spindles conforms to either a simplified or complex pattern. Human spindles, for example, are short, have thin capsules, exhibit inconspicuous equatorial nucleation and have sensory endings that are all of the same simple type, whereas the spindles of artiodactyls such as goat and sheep are more complex (see 14.1).

Recent light-microscope studies of human extraocular muscle spindles (Durston, 1974) have shown no apparent morphological or histochemical differences between the intrafusal muscle fibres, which are not divided into bag and chain fibre types. All intrafusal fibres show high activities for myofibrillar ATPase, oxidative enzymes and non-specific esterases; and low activities for Acid ATPase and phosphorylase. Apart from the low P'ase activity, this profile most closely resembles the C-type profile of the chain fibres in sheep extraocular muscle.

An electron microscope investigation by Mukuno & Nomura (1969) has further shown that the single intrafusal fibre present in a monofibrillar spindle in human extraocular muscle had a 'chain-type' ultrastructure comparable with that of the surrounding extra-

fusal muscle fibres. Although these authors distinguish a second intrafusal fibre type in another spindle with 'poorly-developed intracellular organelles', their illustrations, which are of transverse sections only, are not convincing. The possibility thus remains that the extraocular muscle spindles in man are composed entirely of chain fibres, but perhaps further ultrastructural analysis, particularly of longitudinal sections, will resolve whether this is so, or whether two distinct types of intrafusal fibre are present, as in sheep SR.

In sheep SR the only difference between a nuclear-bag fibre and the surrounding peripheral G fibres is the presence in the former of a typical nuclear bag supplied by a primary afferent that is enclosed in a spindle capsule. All the other features are identical. They possess the same ultrastructure and histochemical profile, receive a common grape motor innervation that is often seen to be derived from the same parent axon, and have grape axon terminals of similar ultrastructure. Similarly, the chain fibre type in SR possesses the same ultrastructure and histochemical profile as its extrafusal counterpart, the small C fibre, with which it shares a common type of motor innervation. The chain fibres may receive both a primary and a secondary sensory supply.

The motor innervation of sheep SR spindles is thus segregated and collateral, grape endings being located on bag fibres and plates on chain fibres. Such spindles might be regarded as small bundles of encapsulated G and C fibres modified by receiving a sensory

innervation consisting of primary and secondary endings.

This pattern of fusimotor supply may be compared to that received by spindles in the fourth toe extensor of frog (see Barker, 1968; 1974; Smith et al.,1973). These amphibian spindles are found almost exclusively in the core of the muscle in close association with one type of plate-innervated 'twitch' fibre and two types of grape-innervated 'slow' fibres. Collateral grape endings are supplied to the thinner of two intrafusal fibre types that has a similar morphology to one of the extrafusal slow fibre types. The twitch motor system provides a collateral plate innervation for the thicker intrafusal fibre and this exhibits a morphology typical of twitch muscle fibres. In contrast to sheep SR, amphibian intrafusal fibre types do not, however, exactly monitor their extrafusal counterparts, either in their ultrastructure (Page, 1966) or their histochemical profile (Smith & Ovalle, 1972; Smith et al., 1973).

The spindles in sheep LP muscle are randomly located in any transverse section and the bag and chain fibres have a similar structure, histochemistry and innervation to those in SR. Since an extrafusal grape-innervated component is absent from the muscle the grape innervation supplied to the bag fibres is exclusively fusimotor, whereas the chain fibres, as in SR, share a collateral plate innervation with extrafusal twitch-type fibres. It appears that in LP the grape-innervated component is restricted to the nuclear-bag fibres of the muscle spindles.

This pattern of fusimotor innervation is somewhat comparable to the fusimotor innervation in those avian and amphibian muscles that are composed almost totally of plate-innervated muscle fibres. The spindles in the posterior latissimus dorsi of chicken (Chin, 1970) and a few spindles in frog sartorius (Sterling, 1974) thus receive collateral plate endings and a grape supply that is purely fusimotor. In frog sartorius these endings are segregated in their distribution to the intrafusal fibre types, as in sheep LP.

This segregation of the motor supply in sheep extraocular muscle spindles, grape endings being located on bag fibres and plate endings on chain fibres, is not maintained in the spindles of the foot muscle studied. In peroneus brevis the multiterminal trail innervation, that might be regarded as the homologue of the grape innervation in the extraocular muscle spindles, has a different juxta-equatorial location within the spindle and is distributed to the typical bag, intermediate bag and chain fibres. Of the plate endings, the p2 type may be distributed to both bag and chain fibres whereas the p1 type has a polar location mainly on bag fibres. This pattern of innervation is comparable to that of cat hindlimb spindles (see Barker, 1968; 1974).

In addition to muscle spindles all three sheep muscles studied contain tendon organs, as is generally the case in mammalian skeletal muscles. In the sheep extraocular muscles the tendon organs are few in number and do not appear to be related to a particular layer of the muscle or to a specific muscle fibre type. Responses from tendon endings, probably tendon organs, have been recorded in goat extraocular muscles (Whitteridge, 1955; Cooper & Daniel, 1957).

They discharge during the phase of increasing muscle tension and although their threshold to stretch is of the same order as that of the spindle afferents, their response to rate of change of stretch is slight.

In extraocular muscles from which typical tendon organs are lacking, it is possible that a similar function is served by the palisade endings (14.32) that terminate at, or near to, the ends of individual muscle fibres. In cat a band of about 30 of these endings extends across the tendon (Huber, 1900). The total number of muscle fibres supplied by palisade endings therefore appears to be of the same order as the number of fibres attached to tendon organs in sheep SR. Barker (1974) supposes that the palisade endings have a similar development to tendon organs except that the initial contact of the afferent nerve fibre with the end of the muscle fibre is maintained. He also discusses evidence that gives support to the concept that palisade endings are associated specifically with the peripheral grape-innervated muscle fibres in the rectus and oblique extraocular muscles. Personal observations on cat extraocular muscles suggest that this may be the case. Even so, it must be borne in mind that conclusive proof is still lacking as to the sensory or motor nature of these endings.

Turning to the functional properties of rectus and oblique extraocular muscles that contain spindles, it has been well established that when a sheep or goat inferior oblique (Cooper, Daniel & Whitteridge, 1951; 1955) or superior oblique muscle (Whitteridge, 1955; Cooper

& Daniel, 1957) is passively stretched, a typical spindle afferent discharge pattern is obtained.

According to Granit (1970, page 251) Fig.1 in Cooper & Daniel's (1957) paper shows the sensitivity of goat extraocular muscle spindles to velocity of stretch, whereas other records (their Fig.3b) indicate so brief a pause after release of stretch that he assumes the presence of static fusimotor activity. However, as yet, the responses of the primary and secondary endings in sheep and goat extraocular muscle spindles have not been distinguished, and the static or dynamic effects of the two types of skeleto-fusimotor axons on these spindle afferents have not been fully established.

It has been suggested (see e.g. Matthews, 1972) that the dynamic response of the primary ending in mammalian spindles arises in terminals that lie on regions of the intrafusal fibres, such as the nuclear bag, that are practically devoid of myofibrils. The presence in sheep extraocular muscle spindles of bag fibres with a comparable ultrastructure to those of spindles from mammalian hind-limb muscles suggests that the collateral grape activation of the bag fibres would be dynamic in function. Similarly, stimulation of the collateral plate endings supplying the chain fibres would presumably have a static effect on the responses of the primary and secondary endings. Matthews & Westbury (1965) found that the collaterals innervating frog spindles had a similar functional identification (grape/dynamic, plate/static), though in these spindles

it is the plate-innervated fibres that are thicker and more modified in the sensory region, with a reticular zone.

The only study of the effect of 'fusimotor' stimulation on the dynamic and static responses of extraocular muscle spindles is that of Whitteridge (1959). He isolated high-threshold axons to the superior oblique of sheep and goats whose stimulation increases the sensitivity of the spindle afferent endings, without appreciably increasing muscle tension. The present study suggests that these axons were skeleto-fusimotor grape axons, the diameter of which is in the 'gamma' range. Evidently the contraction produced in the extrafusal G fibres by stimulation of single skeleto-fusimotor grape axons is weak and produces negligible increase in muscle tension. Since the grape collaterals supply the bag fibres in sheep SR such fusimotor axons might be expected to be dynamic in function. Is there any evidence for this?

For rapid stretches Whitteridge found that the rate of rise and peak frequency of the spindle discharge ' is often identical with and without γ stimulation'. It is likely, because of the extremely rapid stretches used, that the spindle discharge frequency would be maximal, even in the absence of fusimotor stimulation, and would obscure any dynamic effect. In fact Whitteridge mentions that 'with γ stimulation the peak frequency is sometimes a little higher and is usually longer maintained'.

With slower stretches the discharge is accelerated and its maximum value raised by fusimotor stimulation, although Whitteridge stresses that 'these effects are usually smaller than the effect of γ stimulation on maintained discharge'. Approximate readings taken from his Fig.8 indicate that, with slow stretches, fusimotor stimulation increases the dynamic index by a factor of about 2.5, while the maintained discharge during steady stretch is increased by a comparable factor of about 2.6. Since the dynamic index is in fact increased by fusimotor stimulation the axons studied by Whitteridge must be considered to be dynamic in function, and this would accord with the predicted function of the collateral grape endings on the bag fibres.

In spindles with collateral and segregated innervation one might expect that the contractile properties of the intrafusal and corresponding extrafusal fibres would be similar. The bag fibres in sheep SR would thus contract locally without propagating action potentials (similar to the grape-innervated fibres in cat extra-ocular muscles; e.g. Pilar, 1967); and the chain fibres would respond with a twitch contraction. In amphibian spindles, however, Smith (1964a; b) has found that both types of intrafusal fibre are capable of propagating an action potential, although the plate-innervated fibre responds to a single stimulus with a much more rapid contraction. Obviously intrafusal fibres may have different properties from those of the extrafusal fibres with which they share motor axons.

In sheep SR the numerous spindles in the peripheral layers receive a collateral innervation from axons supplying either small C or small/intermediate G fibre types that are probably both slow-contracting, irrespective of their innervation or other physiological properties (see Discussion, Part II). Presumably the contraction of the intrafusal muscle fibres coincides with the activation of some of the slow-contracting extrafusal motor units. Since high spindle densities characterize muscles initiating fine movement, it might also be predicted that the peripheral muscle fibres in sheep SR are responsible for finely-controlled movements that require sensory feedback for their regulation.

In fact, the question of whether the afferent information from the stretch receptors in extraocular muscles, be they spiral endings or muscle spindles, contributes either to extra-retinal position sense or muscle proprioception has been long debated (see e.g. Bach-y-Rita, 1959).

As far as extra-retinal position sense is concerned the 'inflow' theory of Sherrington (1918) has received support from the recent work of Skavenski (1972). He has shown a conscious position sense in the eye muscles of man that can be used to maintain eye position during displacement attempts in total darkness. Although extra-retinal 'inflow' information can be used for oculomotor control, it does not influence the perception of direction (Skavenski, Haddad & Steinman, 1972); for this, 'outflow' information (Helmholtz, 1910) is required.

In the case of extraocular muscle proprioception it is well known that passive stretching of the eye muscles does not produce an increase of tonus characteristic of the stretch reflex of mammalian extensor muscles (McCouch & Adler, 1932; Irvine & Ludvigh, 1936). However, by the use of electromyographic techniques, several types of stretch reflex have been identified in the extraocular muscles of various species. In man (Breinin, 1957) there is a basic static stretch mechanism that cannot be augmented beyond a given point. In rabbit and cat (Baichenko, Matyushkin & Suvorov, 1967) the eye muscles respond to stationary stretching by loads exceeding 2g by developing a 'tonic' stretch reflex. Baichenko et al. propose that this reflex is subserved exclusively by the extrafusal slow muscle fibres that have similar properties to the slow fibres in amphibian muscle. In response to a short vibration stimulus (Marek & Markel, 1971) the eye muscles of cat have recently been reported to exhibit a phasic stretch reflex. Finally, an inhibitory stretch reflex during active contraction has been demonstrated in the cat lateral rectus muscle (Bach-y-Rita, 1972). It is thus apparent that the stretch receptors in extraocular muscle may be involved in both extra-retinal position sense and proprioception, although their precise functional role remains unclear (see e.g. Bach-y-Rita, 1971).

In the present study the previous researches into sheep extraocular muscle spindles have been extended by examining the spindles not as separate entities, but in the context of their

surrounding muscle fibres. In the process it has become evident that extraocular muscles show many resemblances to the zoned limb muscles of Amphibia, both with regard to the organization of their extrafusal muscle fibre components, and also in the manner of innervation of their muscle spindles. It is hoped that the concepts which have emerged will provide a basis for further research and the questions broached stimulate new discussion about the complex problem of eye movements and their control.

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