A COMPARATIVE MORPHOLOGICAL STUDY OF MUSCLE SPINDLES IN THE AVIAN ANTERIOR AND POSTERIOR LATISSIMUS DORSI MUSCLES

by

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ABSTRACT

A study of muscle spindles in two synergistic avian muscles was undertaken to determine whether morphological or quantitative differences existed between muscle spindles residing in a slow-red (tonic) muscle and a fast-white (twitch) muscle. The avian anterior (ALD) and posterior (PLD) latissimus dorsi muscles were chosen since they are unique among vertebrates as paradigms of a slow-red and a fast-white muscle respectively.

Serial frozen sections of muscle were stained with Haematoxylin and Eosin or Gomori trichrome and muscle spindles residing in the ALD were assessed and compared with those in the PLD with regard to organization, distribution and density. Contents of muscle spindles were examined for intrafusal fibre size, number and morphology. Attention was also directed to the relationship between muscle spindles and the surrounding extrafusal muscle in which they were located.

Differences were found between muscle spindles residing in the two muscles. In the slow ALD, muscle spindles were relatively evenly distributed, whereas in the fast PLD, they were concentrated around the single nerve entry point into the muscle. The ALD muscle spindle index was the highest yet published for chicken muscle and was 2.3 times higher than that of its fast counterpart. A bimodal trend in intrafusal fibre diameter was noted in the ALD, and a trimodal trend was found in the PLD. The former had 42% fewer intrafusal fibres than the latter.

Muscle spindles were shorter in the ALD, with an average length of 1.9mm compared with 2.3mm in the PLD. An interesting feature of the slow muscle was the monofibril muscle spindle, containing a single intrafusal fibre.

With a few exceptions, ALD muscle spindles were located within the interfascicular perimysium close to a neurovascular trunk. PLD muscle spindles were rarely seen in these areas but were frequently found within a muscle fascicle, surrounded by closely apposed extrafusal fibres. Moreover, neurovascular trunks were less frequently seen in the PLD.

As an adjunct to this study, three ALD-PLD pairs from the Storrs Connecticut strain of muscular dystrophic chickens were also examined to compare muscle spindles in these muscles with those of normal animals. In the PLD, which is known to exhibit early and progressive pathological change, muscle spindles appeared relatively normal until marked extrafusal fibre degeneration had occured. By this time evidence of muscle spindle involvement included capsular hypertrophy and intrafusal fibre splitting. Whereas the slow ALD has been reported to retain apparent normalcy in muscular dystrophy, subtle changes were seen in some of the muscle spindles examined. These included an increase in number of intrafusal fibres per muscle spindle compared with those in the normal.

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INTRODUCTION

Muscle spindles are complex sensory receptors situated between or within fasciculi of vertebrate skeletal muscles. They contain several components: small specialized intrafusal fibres enclosed by an outer and inner capsule, and both a sensory and a motor innervation. The sensory nerve endings supply the equatorial and juxta-equatorial regions of the muscle spindle, specifically the intrafusal fibres, and the motor nerves supply the contractile poles of these muscle cells. In the equatorial region, the capsule is separated from the intrafusal fibres and their sensory nerve endings by a fluid-filled periaxial space.

The structure and function of mammalian muscle spindles have been studied in detail. Notable reviews have been published concerning the relationship between the central nervous system and the muscle spindle (Granit, 1970; Matthews, 1972), the components of the muscle spindle and their functions (Barker, 1974), characteristics of intrafusal fibres and their innervation (Boyd, 1962) and the functional morphology of intrafusal fibres (Smith and Ovalle, 1972). The majority of studies on muscle spindle morphology and function have been conducted on mammalian muscles, particularly in the cat. Some information is available on amphibian and reptilian species (Barker, 1974), but there is relatively little on avian muscle spindles, and the degree to which they resemble or differ from those of other vertebrates. In addition, there is a scarcity of data on whether muscle spindles reflect characteristics of

the surrounding extrafusal muscle.

In a light microscopic study of the chicken gastrocnemius muscle, De Anda and Rebollo (1967) found that avian muscle spindles were similar in overall structure to those in other species, containing a capsule, a periaxial space, intrafusal fibres in varying number, capillaries and nerve endings. Moreover, position relative to the surrounding extrafusal fibres was found to be parallel, as in mammalian and amphibian species. Three types of intrafusal fibres were observed in this muscle, based on fibre size, arrangement of myofibrils as identified with ferric-haematoxylin, and nuclear configuration in the equatorial regions. Large-diameter intrafusal fibres extended beyond the capsule, and frequently inserted on the perimysium of an adjacent extrafusal fibre. Small-diameter fibres frequently ended within the capsule. An intermediate size of intrafusal fibres was also identified, however not all groups were always seen in each muscle spindle. One or two types of sensory nerve reached the spindle units. Those having a large diameter gave origin to the primary endings on large and intermediate intrafusal fibres, and thin sensory nerve fibres gave origin to the primary endings on small intrafusal fibres. These workers found no evidence of annulo-spiral endings, but described primary endings with wide synaptic contact, and similar but less complex and extensive endings to the small intrafusal fibres. No secondary endings were found innervating these muscle spindles. Two types of motor nerves were observed; thick fibres supplied large-diameter intrafusal fibres at the polar regions, with endings characteristic of motor end-plates, and thinner nerve fibres to small intrafusal fibres, with simpler endings.

A histochemical analysis of the same muscle (Rebollo and De Anda, 1967) further confirmed the presence of three kinds of avian intrafusal fibres. Small-diameter fibres contained large amounts of glycogen in the polar regions, and large-diameter fibres had intense phosphorylase activity and variable glycogen content. Intermediate fibres were negative for phosphorylase with variable glycogen content. All intrafusal fibres showed increased metabolic activity in the equatorial region, associated with their primary sensory innervation and more dense nucleation.

Comparisons between muscle spindles in the sartorius and adductor profundus muscles of four avian species by Maier and Eldred (1971) showed similarities in nuclear aggregation pattern in intrafusal fibres. The "bag" arrangement of nuclei present in mammalian muscle spindles, and described in the bird by De Anda and Rebollo (1967), was not observed by these workers. They found no bimodal or trimodal distribution of intrafusal fibre diameters, nor a consistent pattern of nuclear aggregation. In a subsequent study on the muscle spindles in the flexor carpi ulnaris of the pidgeon (Maier, 1977), a classification of intrafusal fibres by comparing size with intensity of the myofibrillar ATPase reaction was attempted. No constant size relationship was found to correspond with intensity of staining and it was suggested that fibre measurement was of limited value in classifying intrafusal fibres of avian muscle spindles.

In an electron microscopic study of the budgerigar sartorius muscle, James and Meek (1973) confirmed previous light microscopic studies on the avian muscle spindle, indicating that their overall structure was similar to those of mammalian species. No ultrastructural features were

found to suggest the presence of more than one type of intrafusal fibre, nor were nuclear bags seen. These findings were in accord with those of Maier and Eldred (1971) and it was suggested that the degree of overlap of myonuclei reflected different fixation techniques. The outer capsule of the muscle spindle was considerably thinner than that of small mammals, but marked development of the inner capsule was observed. Each intrafusal fibre was surrounded by layers of flattened epithelial cells. Capillaries were occasionally observed within the periaxial space at its maximum diameter, and were separated from the intrafusal fibres by inner capsule cells. No fenestrations were seen in the capillary endothelia. Irregular Z lines of the myofibrils and their lack of M bands, together with a relatively sparse T system suggested that they undergo either slow-twitch or even tonic contractions. This would indicate that the intrafusal fibres of these avian muscle spindles resembled mammalian nuclear bag fibres more than nuclear chain fibres as evidence pointed to a slower rate of contraction in the former than the latter.

In an ultrastructural study of avian muscle spindles in the flexor and extensor hallucis longus, Adal (1973) identified three kinds of intrafusal fibres based on myofilament arrangements , size and number of mitochondria, and amount of sarcoplasmic reticulum. While no segregation of intrafusal fibres into "bag" and "chain" types was seen, it was suggested that these muscle cells might be classified according to the degree to which their fine structure resembled the bag and chain fibres of mammalian muscle spindles. No morphological difference was demonstrated between intrafusal fibres in the flexor from those in the

extensor hallucis longus muscles. The monofibril muscle spindle, containing only one intrafusal fibre, was not found in either muscle, but was mentioned by Maier and Eldred (1971) as being found frequently in avian muscles. Muscle spindles of snakes and lizards also contain only one intrafusal fibre (Fukami, 1970; Pallot and Taberner, 1973).

The fine structure of the muscle spindle capsule in the anterior and posterior latissimus dorsi muscles of the chicken (Ovalle, 1976) was found to consist of an outer portion, composed of multilayered flattened epithelial cells directly continuous with the perineurium, as found in other species (Shantha and Bourne, 1968). It has been suggested that the capsule plays a role as a metabolically active diffusion barrier to the entrance of substances from the external milieu. The inner capsule was implicated in the active synthesis of the amorphous and fibrillar material occupying the periaxial space, in addition to the role of protecting the sensory nerve endings on the intrafusal fibres (Ovalle, 1978).

Studies on vertebrate muscles have indicated a correlation between muscle spindle density, muscle fibre type and muscle function. Richmond and Abrahams (1975a; 1975b) found higher muscle spindle indices in muscles histochemically identified as slow-contracting than in those found to be fast-contracting. In addition, muscle spindles were more numerous in in regions rich in slow extrafusal fibres than in areas containing mainly fast fibres. In a subsequent study on the proprioceptive components of the cat neck, Richmond and Abrahams (1979) discovered high muscle spindle densities in the muscles subserving head and neck movements. These workers also found that muscle spindles were

even more numerous in the small perivertebral muscles, where they were frequently oriented in chains between intramuscular tendons, or clustered in large complexes of 3-10 spindles. Muscle spindles were also found in large numbers associated with myotendon junctions. (Richmond and Abrahams, 1979). Bridgman et al (1962) and Lennartsson (1979) have also shown that mammalian muscles performing finely-co-ordinated movements, muscles of joint stabilization and those containing predominantly slow fibres have a relatively high muscle spindle index. Adal and Chew Cheng (1980) recently examined the number, distribution and density of muscle spindles in two dorsal wing muscles of the domestic duck. Although their sample was small, a relatively high spindle index was found in both muscles, supporting the observations of Maier and Eldred (1971) and James and Meek (1973) that avian muscles frequently have a higher spindle index than is found in mammalian species.

There has been a growing interest in the avian anterior (ALD) and posterior (PLD) latissimus dorsi muscles during the last twenty years, as they are unique paradigms of vertebrate skeletal muscles. It is known that these two muscles differ strikingly in their development (Syrovy and Gutmann, 1967; Wilson et al, 1973; Zelena and Sobotkova, 1973), physiological properties (Ginsborg, 1960a; 1960b; Hnik et al, 1967; Fedde, 1969; Srihara and Vrbova, 1978; Vrbova et al, 1978), morphology (Hess, 1961; Page, 1969; Hess, 1970) and histochemical and biochemical features (Reasons and Hikida, 1973; Wilson et al, 1973; Ovalle, 1978). Moreover, the unique pattern of innervation of the slow, tonic ALD and the fast, twitch PLD has also been demonstrated (Ginsborg, 1960a; 1960b;

Hess, 1961).

The contractile properties of the ALD and PLD differ remarkably both in speed and maintenance of contraction. At 13-16 days of incubation each muscle develops tension and relaxes slowly, but by the 17th day an increase in PLD contraction speed is detectable, and this trend continues until hatching (Gordon and Vrbova, 1975). Sensitivity to acetylcholine is also similar for the two muscles during early embryonic development in that the muscle fibres are sensitive over their whole surface. However after nerve-muscle contact the sensitivity of the PLD decreases while the ALD remains approximately the same (Vrbova et al, 1978). After establishment of neuromuscular junctions the PLD retains this sensitivity only at the motor end-plate, whereas the ALD continues to be sensitive along the plasma membrane to a varied extent (Fedde, 1969; Gordon and Vrbova, 1975).

The avian ALD has a multiple innervation in the form of several small-diameter nerve fibres which terminate in "en grappe" endings on each muscle fibre (Hess, 1961). Slow muscles undergo contracture and do not normally give rise to action potentials (Ginsborg, 1960b; Harris et al, 1973), rather they conduct local potentials between synapses decrementally (Vrbova et al, 1978). The innervation is reported to release insufficient transmitter to initiate action potentials, however Ginsborg (1960b) found small-amplitude action potentials and twitches in several multiply-innervated slow extrafusal fibres in the ALD. Furthermore, Hnik et al (1967) demonstrated propagated action potentials in a small number of ALD muscle fibres following reinnervation by the PLD nerve. Specialization of

the post-synaptic membrane is poorly developed (Hess, 1967; Page, 1969).

The fast-twitch extrafusal fibres comprising the PLD are focally-innervated by one large-diameter nerve fibre with a single end-plate (Hess, 1961). These "en plaque" end-plates are large, relative to "en grappe" endings on slow extrafusal fibres, and unlike in the ALD, have junctional folds. Moreover, they stain intensely for cholinesterase (Fedde, 1969). Motor endings in the slow ALD appear as dispersed droplets, occupy a smaller area of the muscle fibre and stain relatively lightly for cholinesterase (Hess, 1961). Focally innervated muscle fibres undergo a twitch, propagate an action potential, and are capable of high tension development (Hess, 1961); however, they are unable to maintain tension for long periods of time (Page and Slater, 1965). The twitch time for the slow ALD was found by these workers to be 6-7 times slower than that of the PLD.

Slow muscle fibres of the ALD lack a regularly arranged transverse tubular system and sarcoplasmic reticulum such as that of fast muscle fibres (Hess, 1961; Page, 1969; Shear and Goldspink, 1971; Hikida, 1972), and the area of contact between these elements is relatively small (Page, 1969). The ALD obtains its characteristic "felderstruktur" appearance during growth (Shear and Goldspink, 1971; Vrbova et al, 1978). The slow rate of tension development attained by tonic muscle fibres during growth was found to be sufficient to cause rips and deformation of the Z discs, but insufficient to completely split the myofibrils longitudinally. Partial subdivision of the myofibrils was thought to result, leading to lack of extensive development of the sarcoplasmic reticulum (Shear and Goldspink,

1971).

The fast PLD consists almost entirely of twitch or "fibrillenstruktur" muscle fibres (Hess, 1961; 1967). The transverse tubular system and sarcoplasmic reticulum were found by Page (1969) and Shear (1978) to be extensive and well-organized, factors attributed to splitting of the myofibrils into discrete, regular shapes during growth (Shear and Goldspink, 1971). Because of the rapid tension development (Ginsborg, 1960a), the Z discs could rupture and tear, resulting in longitudinal splitting of myofilaments and hence, formation of discrete myofibrils.

Most vertebrate skeletal muscles consist of a mixture of at least two or three fibre types. The slow ALD and fast PLD were considered to have almost exclusive extrafusal fibre homogeneity (Ginsborg, 1960a; Hess, 1970), however in studies of the histochemical characteristics of the ALD (Nene and Chinoy, 1965; Ashmore and Doerr, 1976; Ovalle, 1978) slow extrafusal fibres appeared to be of two varieties. The presence of two morphological and histochemical types of slow fibres occurs in the metapatagialis muscle of the pidgeon (Hikida and Bock, 1974), and in hindlimb muscles of amphibians (Smith and Ovalle, 1973).

There is little information available on the relationship between muscle spindles and the muscles in which they reside. For example, do intrafusal fibres reflect in any way the characteristics of the surrounding muscle ? Ovalle (1978) identified two distinct populations of extrafusal and intrafusal fibres in the avian ALD, and suggested that the histochemical disparity between them may reflect the direct influence of

the sensory innervation on the intrafusal fibres. It would thus be of interest to examine muscle spindles in the avian ALD and PLD because of the predominance of one extrafusal fibre type in each muscle. Because of the known structural, functional, histochemical and biochemical differences between these two synergistic muscles, it is possible that their muscle spindles are also different.

The ALD and PLD have proved useful experimental models in correlation of structure and function. Moreover, the different response of the two muscles to the expression of hereditary muscular dystrophy presents a unique opportunity to investigate factors involved in disease expression and target specificity. The fast, white PLD shows early phenotypic expression of muscular dystrophy whereas the slow, red ALD is spared the effects of the disease (Mazliah et al, 1976; Cosmos et al, 1979a; 1979b; Mazliah and Cosmos, 1979).

Hereditary muscular dystrophy in chickens was first reported by Assmundson and Julian (1956), and described in detail with regard to variability of disease expression in different strains of affected birds by Holliday et al (1968). Several reports have demonstrated morphological similarities of this disease in chickens with human muscular dystrophies (Julian and Assmundson, 1963; Assmundson et al, 1966), however differences of opinion exist as to the relevancy of the chicken as a model for the various forms of human muscular dystrophy. On the other hand, Harris and Slater (1980) emphasize that the dystrophic characteristics common to the various animal species would provide a useful approach to the study of the pathogenesis of the disease.

Relatively little is known about specific pathological changes in muscle spindles since it has been reported that they are disease-resistant in many kinds of muscle pathology (Cooper, 1960; Smith and Ovalle, 1972). Recent attention has been drawn to the involvement of muscle spindles in various mammalian neuromuscular diseases (Cazzato and Walton, 1968; Patel et al, 1968; Meier, 1969; Swash and Fox, 1974; Yellin, 1974), however difficulties were noted with regard to evaluating the discrete pathological changes seen in biopsied material. In a comprehensive study of muscle spindle pathology, Cazzato and Walton (1968) concluded that in human progressive muscular dystrophy the most prominent alterations in the muscle spindles were thickening of the outer capsule and some atrophy of the intrafusal fibres. Unlike in congenital muscular dystrophy, these and other workers noted no direct relationship between the degree of abnormality of the muscle spindle and the severity of extrafusal fibre involvement.

There is no information in the literature on the effects of hereditary muscular dystrophy on the avian muscle spindle. Because the slow-tonic ALD is reported not to phenotypically express the disease, it would seem likely that the muscle spindles would also be spared. Conversely it is possible that the muscle spindles in the dystrophic PLD would undergo pathological change because of the degeneration that is known to occur in the extrafusal muscle. As an adjunct to this study on muscle spindles residing in the normal ALD and PLD muscles, the effects of hereditary muscular dystrophy on the morphology of muscle spindles in the same two muscles will also be considered.

Scope and aims of the present investigation

1. To examine by light microscopic, quantitative, and serial reconstruction methods, muscle spindles residing in two synergistic vertebrate skeletal muscles; the avian anterior (ALD) and posterior (PLD) latissimus dorsi.
2. To ascertain whether morphological and/or quantitative differences exist between muscle spindles residing in the slow-red ALD and the fast-white PLD.
3. To determine the morphological relationships existing between ALD and PLD muscle spindles and the surrounding extrafusal muscle, endomysial connective tissue and neurovascular elements.
4. To examine muscle spindles in the ALD and the PLD of the chicken with hereditary muscular dystrophy and to compare these findings with the normal.
5. To ascertain whether muscle spindles and surrounding tissue in the muscular dystrophic ALD and PLD are affected morphologically in the same or different manner.

MATERIALS AND METHODS

Six male and female white leghorn chickens aged seven weeks, and three muscular dystrophic chickens of the Storrs Connecticut strain, aged nine weeks, were killed with an overdose of chloroform. Body weights were 550-600 grams in the normal and 600-900 grams in the dystrophic birds. The anterior (ALD) and posterior (PLD) latissimus dorsi muscles, used in this study, constitute the most superficial layer of dorsal musculature of the back. These two skeletal muscles are distinctly innervated and remain separate throughout their course, unlike in humans where the two muscles have fused. The strap-like ALD is dark pink in colour. It arises from the neural spines of lower cervical vertebrae and traverses the upper back horizontally to insert as a wide, fleshy semi-tendinous band on the upper medial aspect of the humerus. The PLD is pale in colour and fusiform in shape. It arises from the neural spines of lower thoracic vertebrae and adjacent lumbar fascia, traverses the thorax diagonally and inserts on the humerus as a discrete tendon, slightly ventral to the ALD (George and Berger, 1966).

The right and left ALD and PLD were removed from each animal. Each muscle was trimmed of superficial fascia and tendon, weighed, pinned to a corkboard in a moderately stretched position and moistened with physiological saline. The muscles taken from two normal birds were prepared for paraffin embedding, while the remainder were prepared for frozen sectioning.

The four muscles prepared for paraffin sectioning were immediately fixed in formal saline for four hours, placed in 70% alcohol for 16 hours then dehydrated through ascending alcohols. A modification of the Peterfi method of double embedding (Brown, 1969) was used as it tended to promote easier sectioning. Serial transverse sections were made of each muscle from origin to insertion at a calibrated thickness of 12μ, and every eighth section was mounted on a glass slide. Conventional Haematoxylin and Eosin was used to stain the sections (Lillie, 1965).

Six normal and three dystrophic ALD-PLD pairs were quick-frozen in isopentane-liquid nitrogen and serially sectioned in their entirety in a cryostat at -20° C, at a calibrated thickness of 10μ. Every 10th section was mounted on a glass slide and stained either with conventional Haematoxylin and Eosin or modified Gomori trichrome (Lillie, 1965).

After examination of both paraffin and frozen material with a Leitz Orthoplan microscope, it was found that the quick-freezing technique had preserved the tissue with minimal artefact. Muscle spindles were easily identified and their contents could be counted, assessed and measured. The paraffin technique involved considerably more tissue distortion and, as a result, many muscle spindles were either difficult to identify or their contents appeared clumped in one area of the capsular space (Fig. 13). As this prevented accurate counting and measurement of intrafusal fibres, it was decided to use the paraffin sections only as an adjunct to the frozen material. Accordingly, an examination was made of six normal ALD-PLD pairs using the following protocol.

Muscle spindles were located by microscopy at x400 magnification,

numbered, and each equatorial region was marked on a scale drawing of the muscle cross-section using the micrometer scale readings on the mechanical stage of the microscope. Repeat procedures were done to check for error. Spindle units were then marked on a scale drawing as they appeared in the serial sections, and the approximate length of each was calculated by counting the number of cross-sections in which the muscle spindle was seen, and multiplying this by a factor of 10. Extracapsular and intracapsular polar, juxta-equatorial and equatorial regions were also noted and their respective lengths calculated.

The muscle spindle index (number of muscle spindles per gram of muscle weight) was calculated for the six normal ALD-PLD pairs, and comparisons were made with the results collected from published data on spindle indices from other avian muscles and from those of other species. Longitudinal scale drawings of one normal ALD-PLD pair were made from serial tracings (Gaunt and Gaunt, 1978) utilising the microscope equipped with a Leitz camera lucida drawing attatchment. Both muscles were serially reconstructed from the traced sections. Included in each tracing were outlines of muscle spindle capsules, extracapsular polar regions, number of intrafusal fibres per spindle unit, and location of prominent neurovascular elements. The longitudinal reconstructions gave a fairly accurate representation of muscle spindle size, location and length relative to myotendon junctions and neural elements. In addition, a transverse plane representation of the two muscles was made from tracings taken at 4mm intervals in order to illustrate the muscle spindle positions relative to ventral and dorsal surfaces. Histograms were constructed from tracings

taken at 2mm intervals to show the degree of uniformity of spindle distribution in the two muscles.

Intrafusal fibres were counted from 100 ALD and 100 PLD muscle spindles and the range of fibres per spindle unit were depicted in histograms. A total of 250 of these intrafusal fibres from the ALD and PLD were also measured in juxta-equatorial regions. Fibre size (cross-sectional diameter) was calculated by taking the average of two readings at right-angles and these results were depicted in companion histograms. A similar process was used to demonstrate extrafusal fibre diameters in the two muscles. A total of 150 extrafusal fibres from the mid-belly region of the ALD and its PLD counterpart were measured, and the results shown in a similar manner as that undertaken for the intrafusal fibres.

Finally, three dystrophic ALD-PLD pairs were examined and compared with the normal sample in order to assess any detectable morphological alterations in muscle spindles and their surrounding extrafusal muscle. One of the three pairs was examined in detail for location, distribution and appearance of muscle spindles, number and size of intrafusal fibres and muscle spindle index. Random sections from the origin, mid-belly and insertion of the remaining ALD-PLD pairs were used for additional morphological data.

RESULTS

Comparative data from quantitative analysis of muscle spindles in six normal ALD-PLD pairs, and of each muscle weight, are shown in Table 1. The mean weight of the ALD was 0.279gm, consistently less than that of the PLD with a mean weight of 0.316gm. A total of 315 muscle spindles were counted and examined; 212 and 103 from the ALD and PLD respectively. The range of muscle spindles per muscle was 32-40 in the ALD with a mean of 35.33, and 16-20 in the PLD with a mean of 17.16. Muscle spindle indices for the six ALD-PLD pairs is shown in Table 1. This important measurement indicates the number of muscle spindles per gram of muscle weight. It was significantly higher in the ALD (mean = 130.33), than in the PLD (mean = 56.14).

The light microscopic appearance of muscle spindles observed in this study was similar in many respects to that described for other avian muscles (De Anda and Rebollo, 1967; Maier and Eldred, 1971). In the present study, however, several differences were observed between muscle spindles residing in the ALD and those present in the PLD. Figure 1 shows the overall appearance of the two muscles in transverse section and the typical location of muscle spindles. The ALD (Fig.1a) shows two muscle spindles located at the periphery of discrete muscle fascicles close to a neurovascular trunk. The PLD (Fig.1b) shows a muscle spindle partly enclosed in a fascicle, some distance from neurovascular elements. Perimysium

is less evident here than in the ALD, with a relatively larger number of extrafusal fibres contained in each fascicle.

The distribution and individual lengths of all muscle spindles in an ALD-PLD pair is shown diagramatically in Figure 2. The ALD has a relatively even distribution of spindle units located from origin on the vertebral column to insertion on the proximal end of the humerus. A slight increase in spindle density appeared to occur in the region of entry of each of the three branches of the median nerve into the muscle. On the other hand, a relatively uneven distribution of spindle units was noted in the PLD with the majority concentrated in the distal third of the muscle, around the entry point of the single branch of the median nerve which supplied it. In three of the six PLD muscles used in this study, only one muscle spindle was found in the proximal third of each muscle belly.

Muscle spindle lengths, including extracapsular polar regions, were shorter in the ALD, with a range of 0.8-3.7mm (mean = 1.9mm) compared with a range of 1.1-4.8mm (mean = 2.3mm) in the PLD. The majority of intrafusal fibres were seen to extend beyond the capsule in both the ALD and PLD and these fibres terminated within a short distance of each other. The small monofibril muscle spindles found only in the ALD were rarely longer than 1mm. The longest muscle spindles were usually found in the PLD, frequently extending 1mm beyond the limit of the capsule at each pole.

Figures 3 and 4 show the density of muscle spindles per transverse section at 2mm and 4mm intervals, respectively. In Figure 3, nerve entry points correspond approximately with intervals 4,6 and 9 in the ALD and between intervals 6 and 8 in the PLD. In Figure 4 it can be seen that

muscle spindles are more frequently located on the ventral aspect of each muscle. It was also noted that the nerve supply entered each muscle on the ventral aspect. Moreover, at least one muscle spindle was observed in the region of the myotendon junction at the insertion of both muscles.

Polar regions of muscle spindles from the two muscles are shown for comparison in Figure 5. Intrafusal fibres were usually scattered within the endomysium of surrounding extrafusal fibres in the extracapsular regions in the ALD (Fig.5a), while they were compactly arranged within the endomysium in the PLD (Fig.5b). Moreover, ALD muscle spindles were usually isolated from surrounding muscle by prominent connective tissue (Fig.5c), while those in the PLD were more intimately related with adjacent extrafusal muscle fibres (Fig.5d). Juxta-equatorial muscle spindles in the two muscles are seen in Figure 6. A similar relationship between connective tissue and the capsule as found in the intracapsular polar spindles is present in the ALD (Fig.6a). An intimate relationship between capsule and extrafusal fibres is again seen in the PLD (Fig.6b). Inner capsule cells and a fluid-filled periaxial space are present in the ALD and PLD muscle spindles with a more darkly-staining extracellular material present in the periaxial space of the latter. This difference in staining intensity can be seen in examples of equatorial regions of ALD and PLD muscle spindles in Figure 7. As can be seen, the ALD contains several intrafusal fibres and inner capsule cells lying within a lightly stained periaxial space, surrounded by an attenuated outer capsule. In contrast, the PLD has a marked abundance of inner capsule cells and a more darkly stained periaxial space. Whereas the relationship between the capsule of the PLD muscle spindle and

surrounding extrafusal fibres remains approximately the same in Figures 5, 6 and 7, the ALD counterpart shows a decrease in connective tissue towards the equatorial region. In Figure 7, therefore, the relationship between ALD and PLD muscle spindle capsules and surrounding extrafusal muscle appears similar. The lack of noticeable increase in nucleation of intrafusal fibres is apparent in both examples, thus confirming previous observations in other avian muscle spindles (Maier and Eldred, 1971; Adal, 1973; Maier, 1977). The primary sensory innervation, which enters the equatorial region, can be seen surrounding several intrafusal fibres (Fig.7).

Conjunctive forms of muscle spindles include all those which either share elements or which lie in close proximity with other units (Richmond and Abrahams, 1975b).Only one tandem or series linkage was seen in this study, and it occured in the distal third of a PLD (Fig.2). Paired muscle spindles have some form of mechanical contact without sharing intrafusal fibres (Barker and Ip, 1961). Several examples of these were found in the ALD sample. Figure 8a shows two adjacent and parallel muscle spindles with adherent but separate external capsules. The smaller of the two units is monofibril, in that it contains only one intrafusal fibre. Figure 8b shows such a monofibril muscle spindle at higher magnification, This section through the equatorial region illustrates the presence of a periaxial space, inner capsule cells and a sensory innervation to the single intrafusal fibre. Another muscle spindle pair with parallel external capsules lying adjacent to each other is found in Figure 8c.

Attempts to classify avian intrafusal fibres according to size

have usually been unsuccessful (Maier and Eldred, 1971; Adal, 1973; Maier, 1977). In the present study, however, measurement of these muscle cells in the ALD and PLD showed differences in number of intrafusal fibres per muscle spindle and in intrafusal fibre diameter. Table 2 shows the range of intrafusal fibre number measured in 212 ALD and 103 PLD muscle spindles. From the ALD sample the range of intrafusal fibres per spindle unit was 1 - 8 with an overall mean of 3.45. In the PLD sample the intrafusal fibre range was 2 - 9 with an overall mean of 4.91. A total of 24 monofibril muscle spindles was found in the ALD sample, with a minimum of three and a maximum of eight per muscle. From a total of 100 ALD and 100 PLD muscle spindles, the number of intrafusal fibres per muscle spindle is shown graphically in Figure 9. Note that the maximum number of ALD muscle spindles (n = 21) contained three intrafusal fibres, compared with five intrafusal fibres in the maximum number of PLD spindles (n = 26).

Measurement of intrafusal fibre diameters was undertaken in juxta-equatorial regions to avoid incorporating the primary sensory innervation into the reading (Boyd, 1962). Measurements were taken from a total of 250 ALD and 250 PLD intrafusal fibres and their distribution is graphically depicted in Figure 10. Intrafusal fibre diameters in the ALD ranged from 5 to 16.5μ (mean = 10.11μ) compared with a range of 4.5 - 18.5μ (mean = 10.71μ) in the PLD. A bimodal distribution in intrafusal fibre size in the ALD with peak values of 8μ and 10μ, and a trimodal trend in the PLD with peak distribution between 7 - 8μ, 9 - 10μ and 11 - 12μ indicate the possibility of two types of intrafusal fibres in the ALD and three in the PLD.

Extrafusal fibre diameters were also measured from the mid-belly region of the two muscles and the results are shown in Figure 11. The range of extrafusal fibre size in the ALD was 28 - 57μ (mean = 43μ) compared with a range of 16 - 43μ in the PLD (mean = 31μ). It can also be seen in Figures 1 and 12 that extrafusal fibres in the ALD a larger than those in the PLD. In Figure 12, moreover, a prominent capsule surrounding the ALD muscle spindle and a less distinct capsule of the PLD muscle spindle were also observed.

Paraffin material was also used as an adjunct to the frozen sections examined in this study. Figure 13 represents paraffin sections through different regions of ALD and PLD muscle spindles. It can be seen that extrafusal fibres, external capsules and contents of muscle spindles are less well-defined in the paraffin material than in the frozen sections.

One ALD-PLD pair was obtained from a chicken with muscular dystrophy, and analysed in the same manner as the normal sample, except for the omission of the longitudinal and transverse reconstruction procedures. Whereas the light microscopic appearance of the majority of muscle spindles in the dystrophic PLD was normal, some pathological changes were observed in several spindle units. Widespread changes in PLD extrafusal fibres were also found. In the dystrophic ALD counterpart subtle changes were seen in muscle spindle characteristics but the extrafusal muscle appeared normal.

Figure 14a shows a dystrophic PLD with irregularly arranged extrafusal fibres, many of which are either considerably larger or

markedly smaller than the normal. Connective tissue has infiltrated between many of the fibres, giving the appearance of more discrete separation of the muscle fibres into fascicles. Myofibrillar clumping, indicated by increased intensity of Eosin stain, was also seen in several muscle fibres. Muscle spindles frequently appeared normal despite surrounding extrafusal fibre degeneration. Figure 14b shows such an example from the dystrophic PLD. The intrafusal fibres appear intact, however the external capsule has thickened.

A total of 41 dystrophic muscle spindles were counted in a muscle pair; 28 in the ALD and 13 in the PLD. Table 3 compares data from this dystrophic ALD-PLD pair with the results obtained in the six normal muscle pairs. Although the mean weight of the normal sample was not directly compared with the dystrophic material because of a two-week age difference, it is interesting to note that the ratio of the mean normal ALD-PLD weights was 0.9 : 1, compared with 1.57 : 1 in the dystrophic sample. Data obtained from the two remaining dystrophic ALD-PLD pairs also confirmed this apparent weight increase in the dystrophic ALD and marked weight loss in its PLD counterpart.

The muscle spindle index in the dystrophic ALD (79.86) was notably lower than that seen in the normal ALD sample (130.33) (Table 3). While the dystrophic PLD counterpart also contained fewer muscle spindles than that observed in the normal sample, the spindle index was appreciably higher in the dystrophic muscle (58.27) than in the normal sample (Table 3). It is known that the full complement of muscle spindles is attained by each muscle shortly after birth (Zelena, 1957;

Shear and Goldspink, 1971; Barker, 1974), therefore a dystrophic muscle such as the PLD may show such an increase in the muscle spindle index as atrophy and weight loss occur.

A juxta-equatorial muscle spindle from the dystrophic PLD is seen in Figure 15a. Several abnormalities are present in the form of extrafusal fibre atrophy, centrally placed nuclei, hypertrophied external capsule of the muscle spindle and apparent intrafusal fibre splitting. Two adjacent intrafusal fibres are less than 3μ in diameter, approximately half the diameter of the smallest intrafusal fibres in the normal PLD sample, and it is tempting to speculate that they were originally a single fibre.

The apparent normalcy of ALD extrafusal fibres as reported previously (Cosmos et al, 1979; Mazliah and Cosmos, 1979), is confirmed in the present study. However, some changes were noted in the appearance of several dystrophic ALD spindles. There were no monofibril units observed. In addition, the number of intrafusal fibres per muscle spindle had apparently increased considerably (Table 3). This value was higher in the dystrophic ALD than in the PLD in both range and mean. Figure 15b shows a dystrophic ALD equatorial muscle spindle containing 11 intrafusal fibres. Several other equally large units were found in the dystrophic ALD material, whereas in the normal sample only two or three spindles per muscle, with a maximum of 7 or 8 intrafusal fibres, were seen. The range of PLD intrafusal fibres per dystrophic muscle spindle (Table 3) was the same as that observed in the normal sample, and the small increase in the mean (5.00 compared with 4.91) may be due to some intrafusal fibre splitting.

Table 1. A tabulation of the number and density of muscle spindles in a total of 6 normal ALD-PLD pairs. Individual muscle weights are also indicated and were used to compute the muscle spindle index.

Muscle	Weight in grams	Muscle Spindle content	Muscle spindle index
ALD	0.183	32	175.05
PLD	0.212	17	80.14
ALD	0.282	32	113.33
PLD	0.344	17	47.05
ALD	0.272	36	131.37
PLD	0.342	16	46.88
ALD	0.363	40	110.26
PLD	0.401	20	49.85
ALD	0.322	40	124.30
PLD	0.399	16	47.14
ALD	0.251	32	127.49
PLD	0.258	17	65.82
ALD mean	0.279	35.33	130.33
PLD mean	0.316	17.16	56.14
ALD: PLD (ratio means)	1:1.133	2.06:1	2.32:1

Table 2. Data illustrating the range of intrafusal fibres per muscle spindle in the six ALD-PLD pairs. The ALD-PLD ratio of means has been computed and is indicated on the extreme right.

Muscle	Range	Mean	ALD:PLD
ALD	1-8	3.14	1:1.54
PLD	3-7	4.83	
ALD	1-7	3.13	1:1.57
PLD	3-7	4.90	
ALD	1-8	3.71	1:1.30
PLD	2-7	4.83	
ALD	1-8	4.26	1:1.18
PLD	2-9	5.00	
ALD	1-7	3.43	1:1.43
PLD	2-7	4.89	
ALD	1-8	3.00	1:1.63
PLD	2-6	4.89	
		Total Mean	1:1.43

Table 3. Summary of salient data comparing dystrophic (n = 1) and normal (n = 6) ALD - PLD muscle pairs. The data indicated for normal muscles represents means.

	Dys. ALD	Dys. PLD	Normal ALD	Normal PLD
Muscle Weight grams.	0.351	0.224	0.287	0.316
Muscle Spindle Number	28	13	35.33	17.16
Muscle Spindle Index	79.86	58.27	130.33	56.14
Intrafusal fibres per muscle spindle	range 2 - 11 mean 5.10	2 - 9 5.00	1 - 8 3.45	2 - 9 4.91

Figure 1

a. Transverse frozen section of the normal ALD. Extrafusal muscle fibres are arranged into discrete fascicles. Two muscle spindles (arrows) are located in close proximity to prominent neurovascular elements. H and E stain. X100

b. Transverse frozen section of the normal PLD. Whereas the extrafusal muscle fibres are smaller than in the ALD, here they are arranged into fascicles with a larger cellular population. A muscle spindle (arrow) is seen at a further distance from neurovascular elements. H and E stain. X100

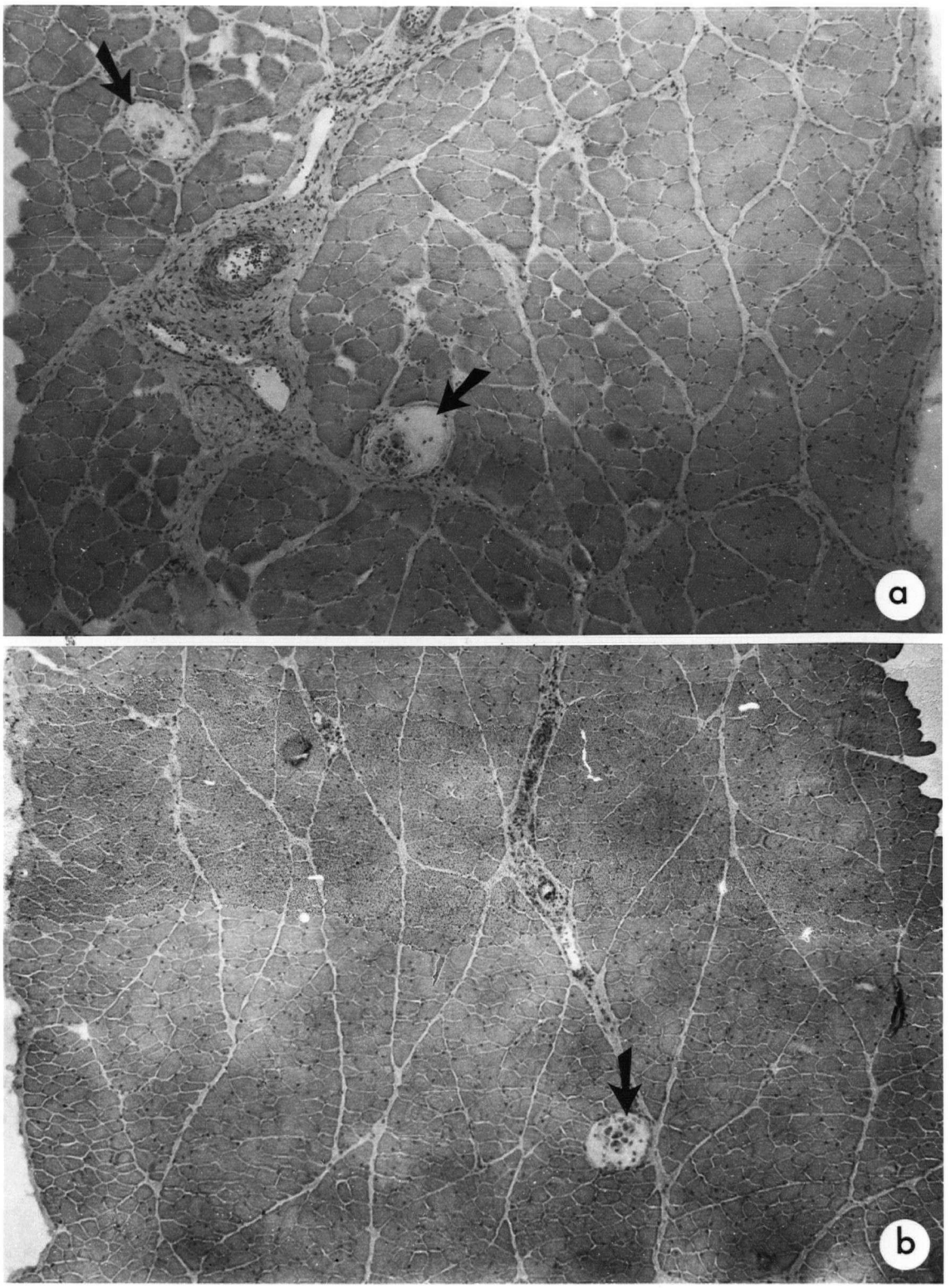

a
b

Figure 2

Longitudinal reconstruction of an ALD and a PLD showing the distribution and lengths of muscle spindles in each muscle. Lengths of equatorial regions are indicated by thickened lines. Rostral (r), caudal (c), origin (o), insertion (i).

Scale: 1mm = 100μ.

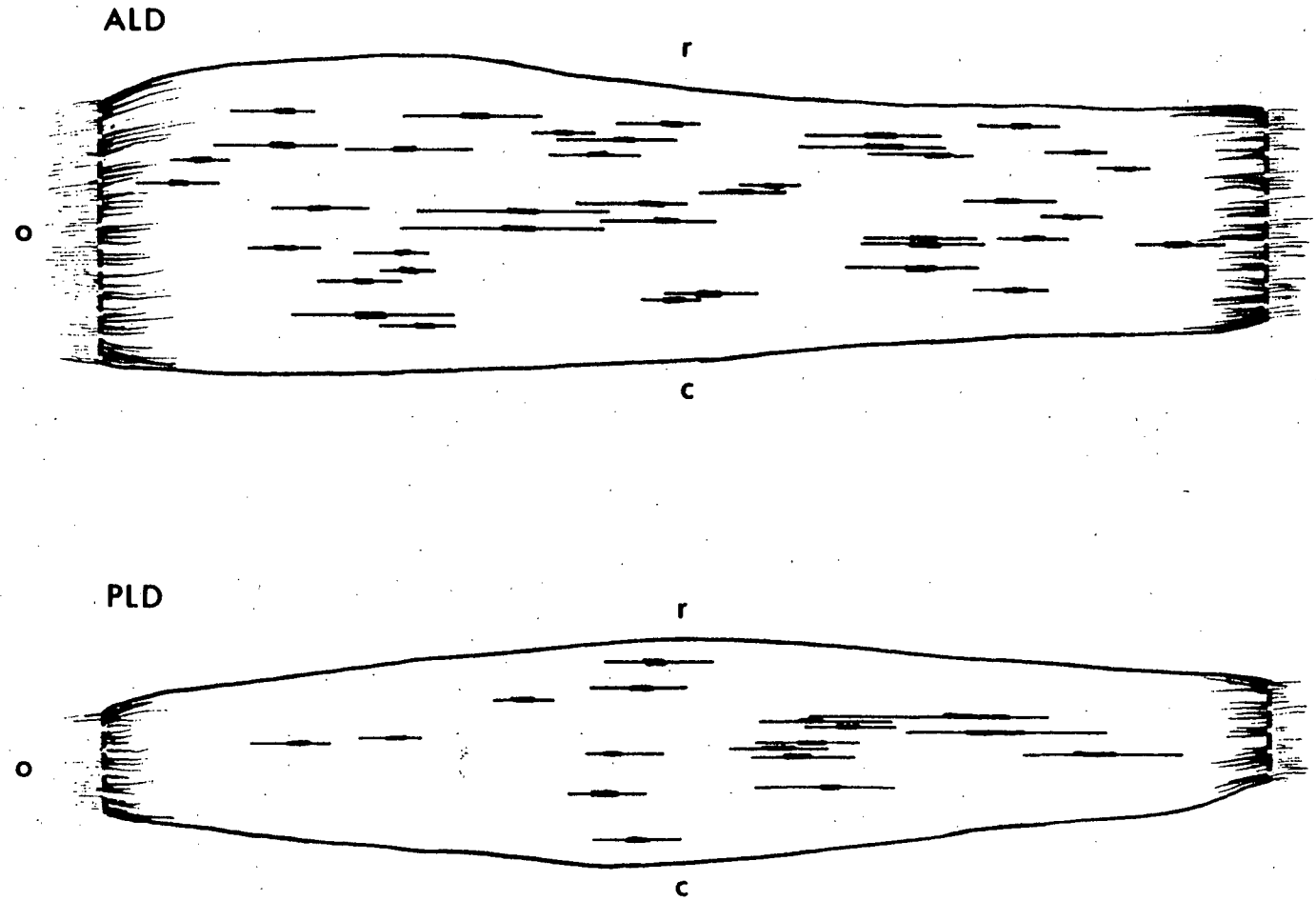


Figure 3

Histogram illustrating the number of muscle spindles observed at 2 mm intervals from origin to insertion of one ALD (left) and its companion PLD (right).

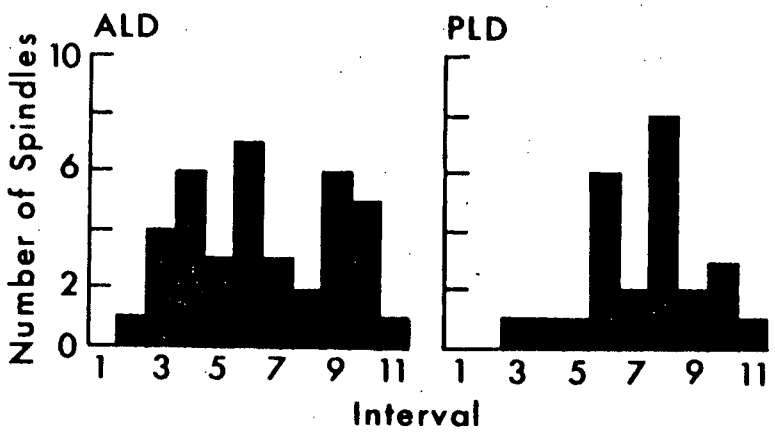


Figure 4

Transverse reconstruction showing the distribution of muscle spindles in one normal ALD-PLD pair from origin to insertion. Each dot indicates a muscle spindle. Intervals between traced transverse sections are 4mm.

Rostral (r), caudal (c), ventral (v), dorsal (d).

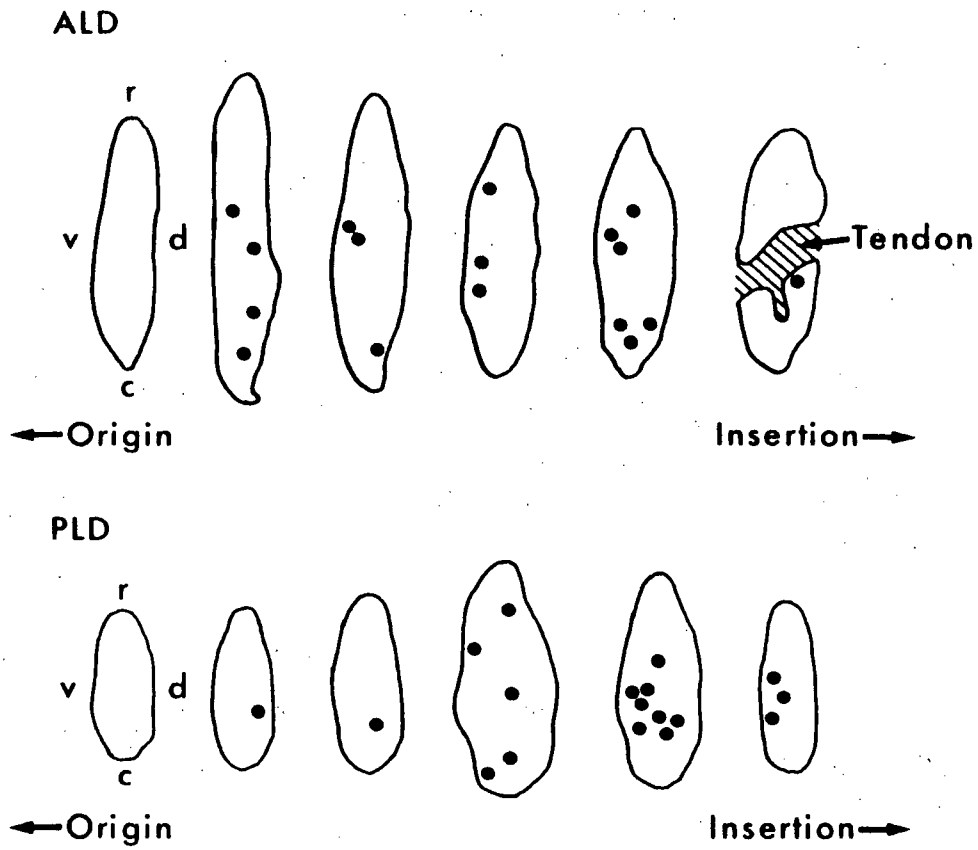


Figure 5

Transverse frozen sections of polar regions of muscle spindles from the ALD (a, c) and the PLD (b, d). H and E stain. X1,000

a. ALD. Several extracapsular intrafusal fibres are visible between neighbouring extrafusal fibres.

b. PLD. Several small extracapsular intrafusal fibres are more tightly arranged within the endomysium of the surrounding extrafusal fibres.

c. ALD. A capsule (arrow) intimately surrounds the intrafusal fibres in the polar region. Endomysial connective tissue associated with the capsule is conspicuous.

d. PLD. In this muscle, a muscle spindle capsule (arrow), investing the intrafusal fibres, is associated with minimal amounts of endomysial connective tissue.

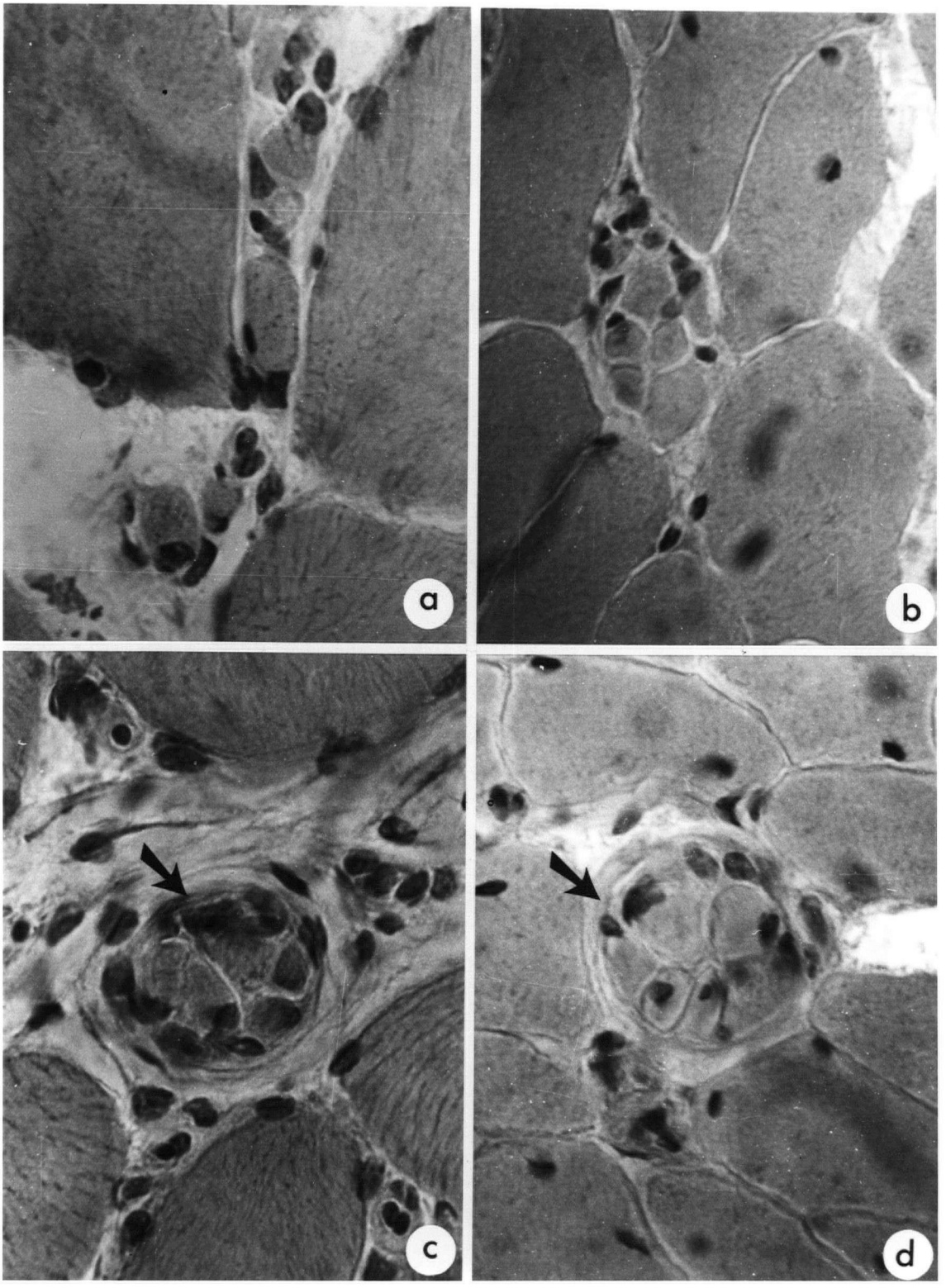

a
b
c
d

Figure 6

Transverse frozen sections of juxta-equatorial regions of muscle spindles in the ALD (a) and the PLD (b) for comparison. H and E stain x1,000. The ALD muscle spindle capsule (arrow) is surrounded by an abundance of connective tissue. The PLD exhibits less connective tissue between its muscle spindle capsule (arrow) and the extrafusal fibres. Two of the three intrafusal fibres in the PLD appear larger than their counterparts in the ALD.

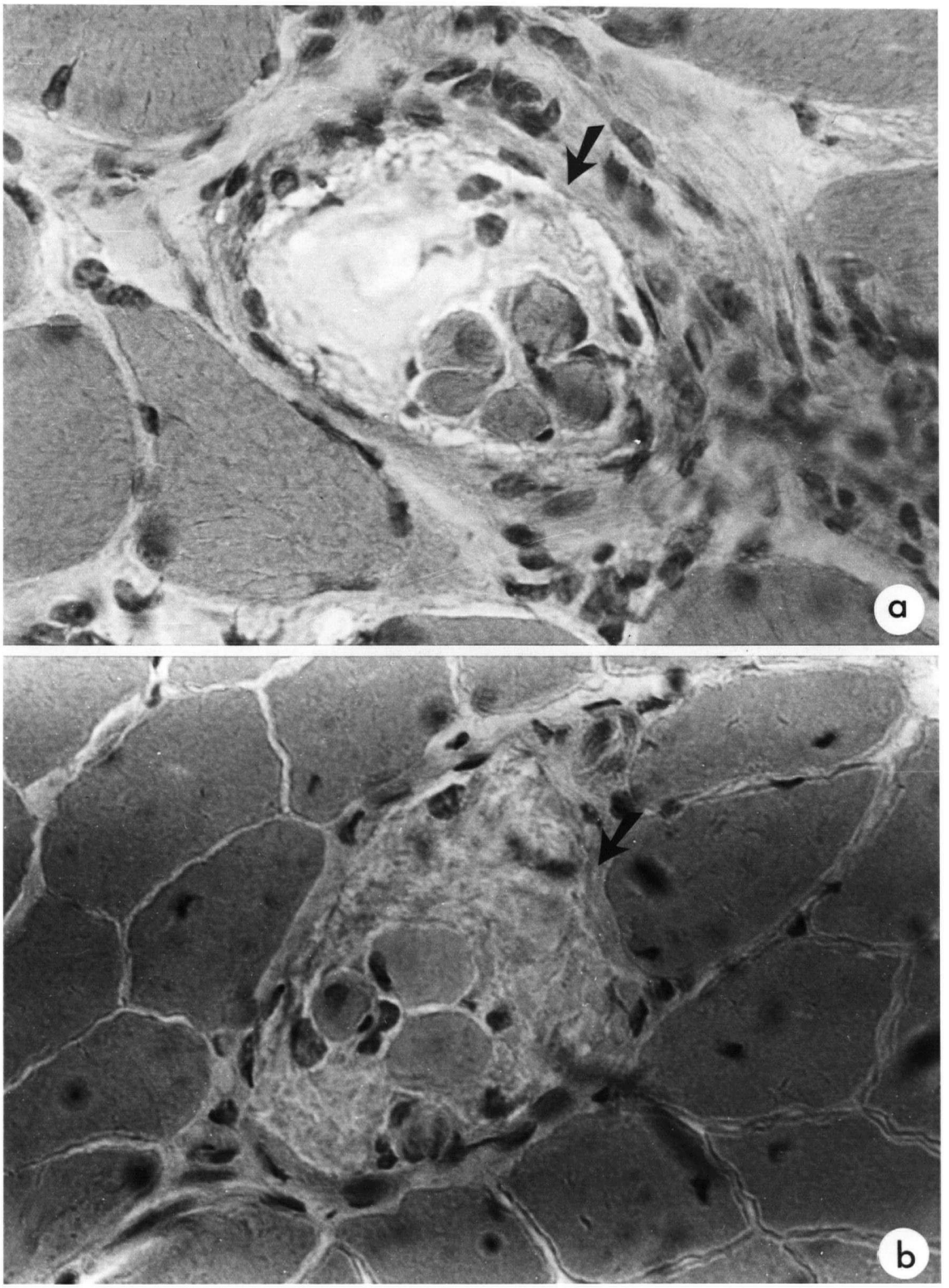

a
b

Figure 7

Transverse frozen sections of equatorial regions of muscle spindles in the ALD (a) and PLD (b). H and E stain. X900. In the ALD, note the sensory terminal (small arrow) on one of the five intrafusal fibres, and the arrangement of inner capsule cells (arrowhead). In the PLD, sensory terminals surrounding the intrafusal fibres are visible (small arrows). Here, a large number of inner capsule cells (arrowhead) are distributed throughout the periaxial space. The outer capsules (curved arrows) and adjacent extrafusal fibres (E) are indicated.

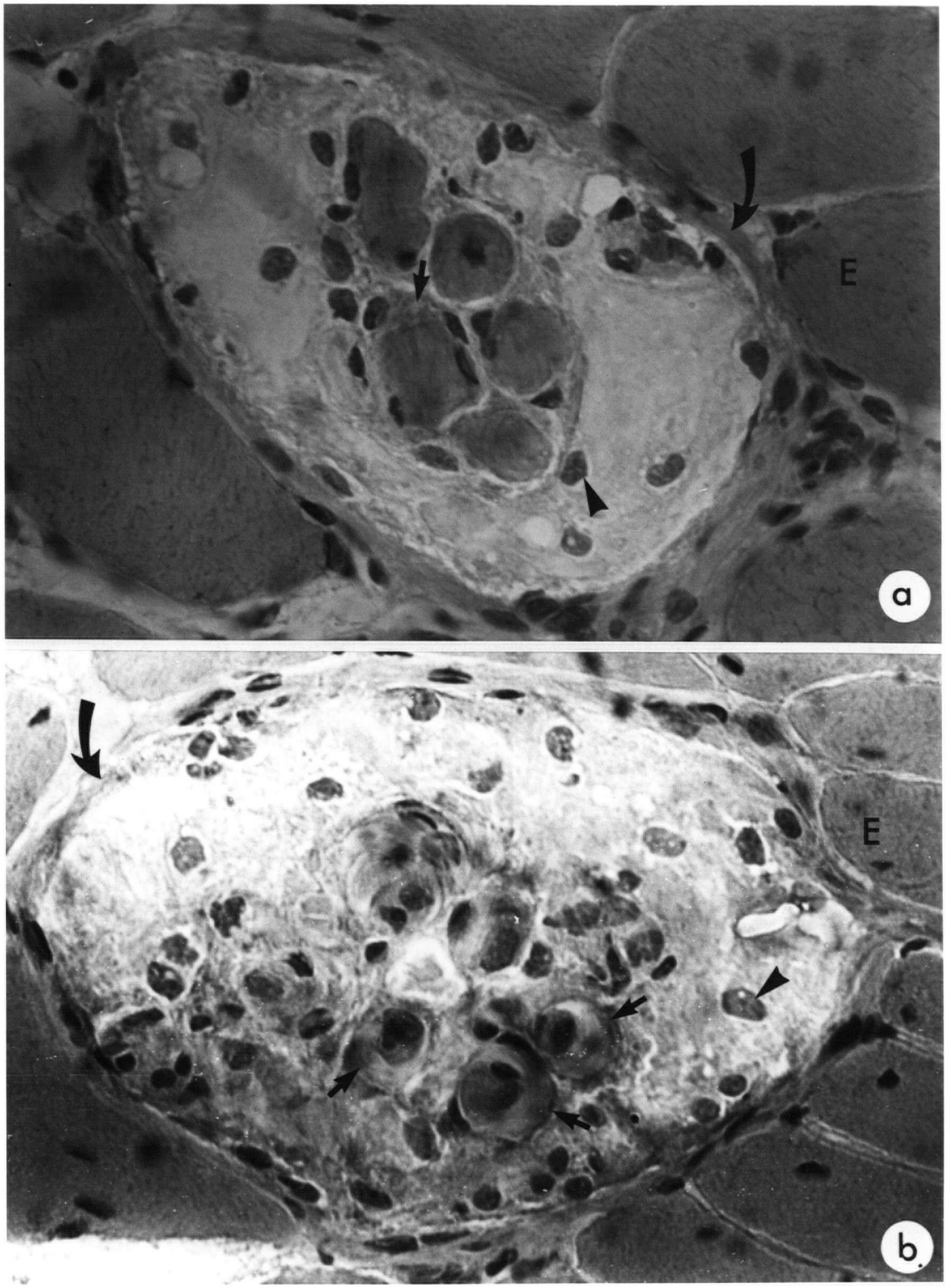

E
a
E
b

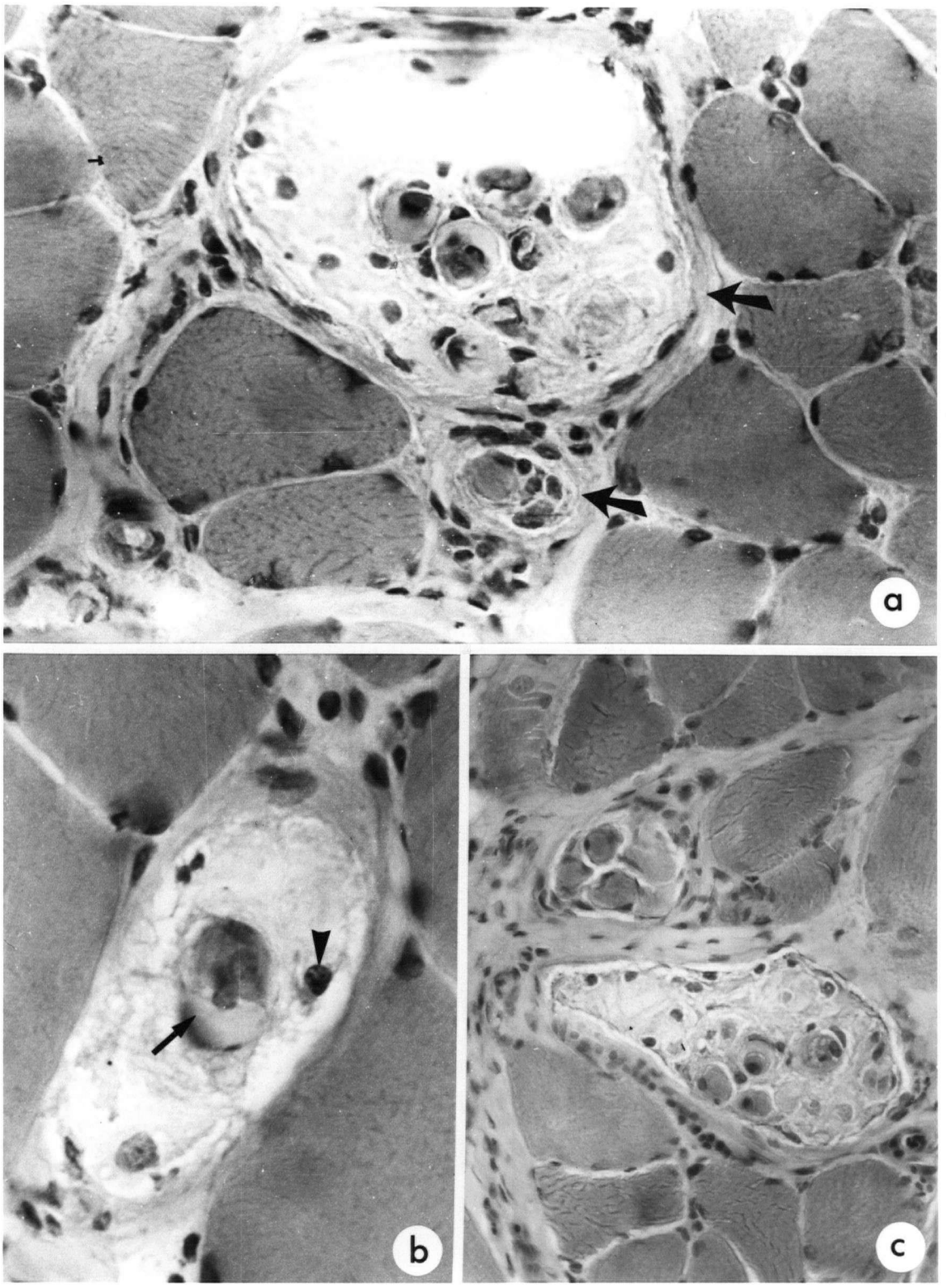
Figure 8

Transverse frozen sections of several ALD muscle spindles stained with H and E.

a. Two parallel muscle spindles are seen in which their outer capsules (arrows) adhere but remain separate and distinct. The equatorial muscle spindle contains five intrafusal fibres and lies within the same section of endomysium as the monofibril muscle spindle below. X750

b. High magnification view of the equatorial region of a monofibril muscle spindle. A sensory terminal (arrow) on the intrafusal fibre and an inner capsule cell (arrowhead) are visible. X1,000

c. Section of two ALD muscle spindles situated on the periphery of adjacent fascicles. The polar muscle spindle (above) contains five intrafusal fibres, and the equatorial muscle spindle (below) contains four intrafusal fibres. X430


a
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c

Figure 9

Graphic representation of the total number of intrafusal fibres per spindle existing in a sample of 100 ALD and 100 PLD muscle spindles.

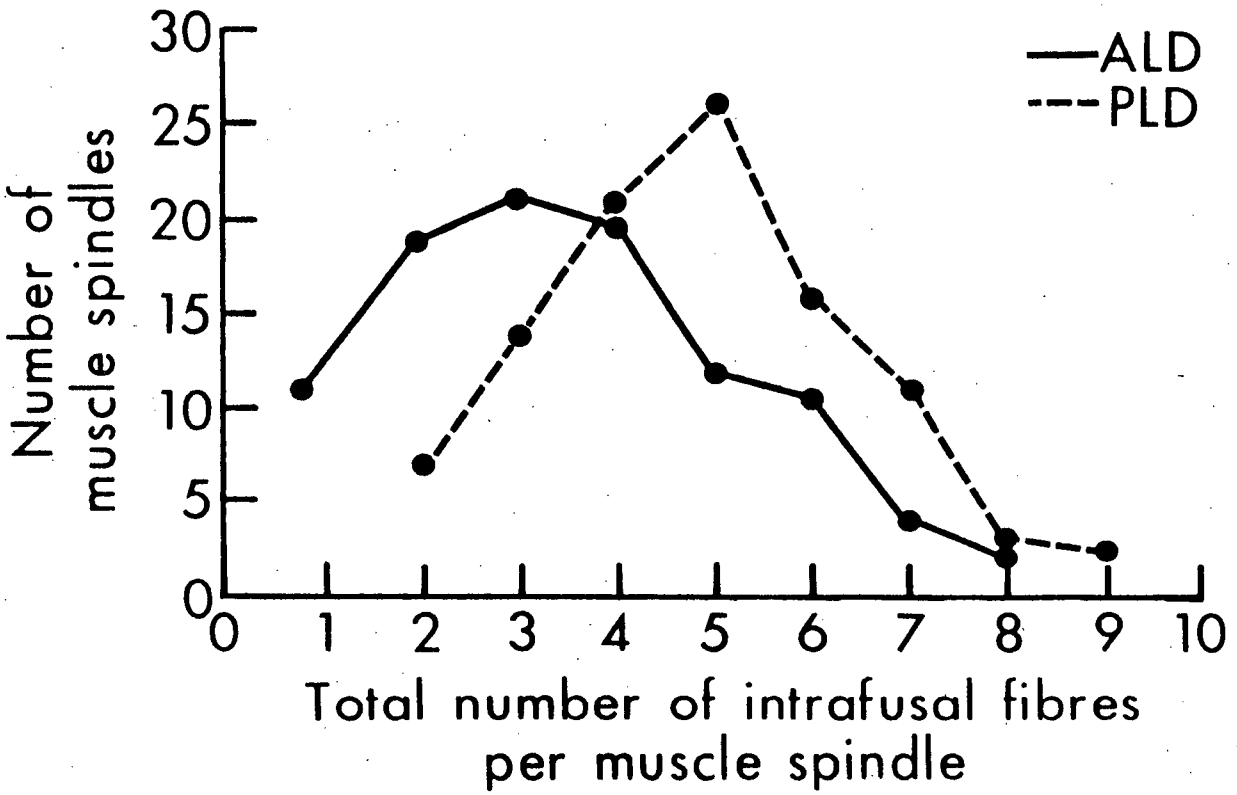

Figure 10

Size histograms of intrafusal fibres from selected muscle spindles in the ALD and the PLD. A total of 250 fibres from each muscle were measured, and this data was collected from six normal ALD-PLD pairs. Note the apparent bimodal distribution of cross-sectional diameters in the ALD, and a trimodal trend in the PLD.

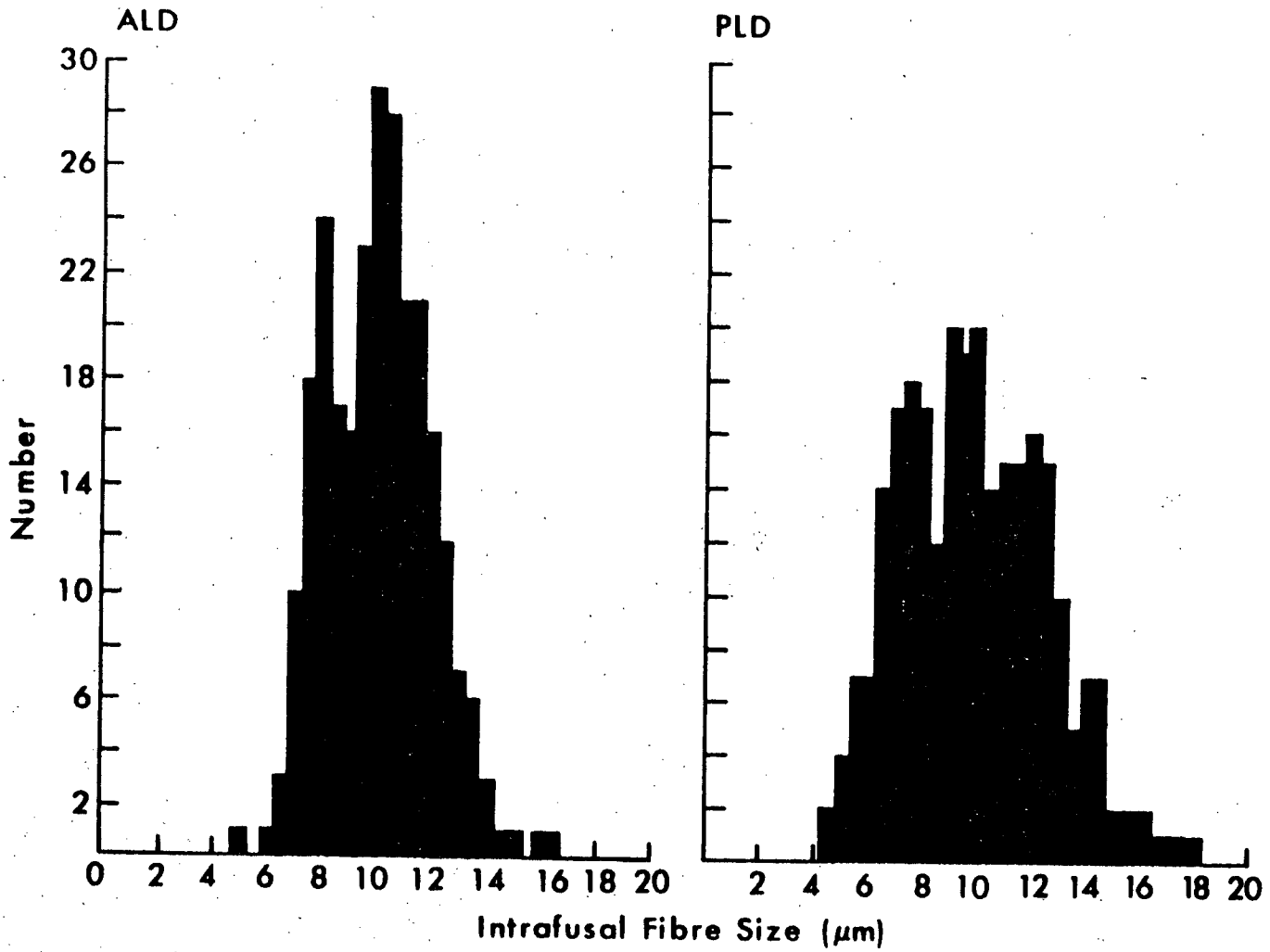

Figure 11

Size histograms of extrafusal fibres in the two muscles. A total of 150 fibres were selected randomly from the midbelly regions of each muscle.

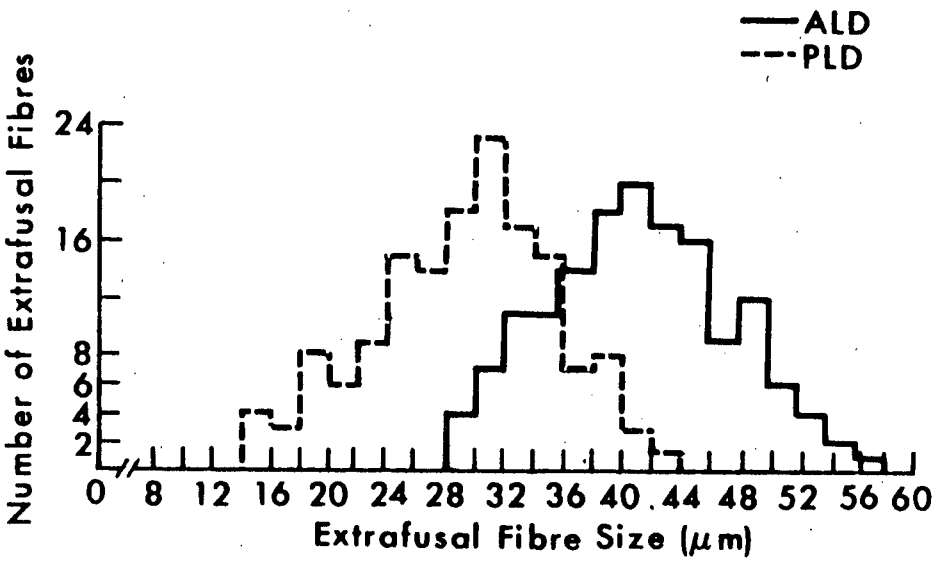

Figure 12

a. Transverse frozen section of an ALD equatorial muscle spindle. Gomori trichrome stain. Five intrafusal fibres and their associated sensory terminals are evident. Note the lack of a well-defined clustering of nuclei within the intrafusal fibres. There is a prominent outer capsule separating the muscle spindle from adjacent extrafusal fibres. X750

b. Transverse frozen section of a PLD equatorial muscle spindle. Gomori trichrome stain. The lack of a "bag-type" nuclear configuration in any of the six intrafusal fibres is similar to that seen in the ALD. The outer capsule is less prominent. Note also the relatively small size of PLD extrafusal fibres compared with those in the ALD. X750

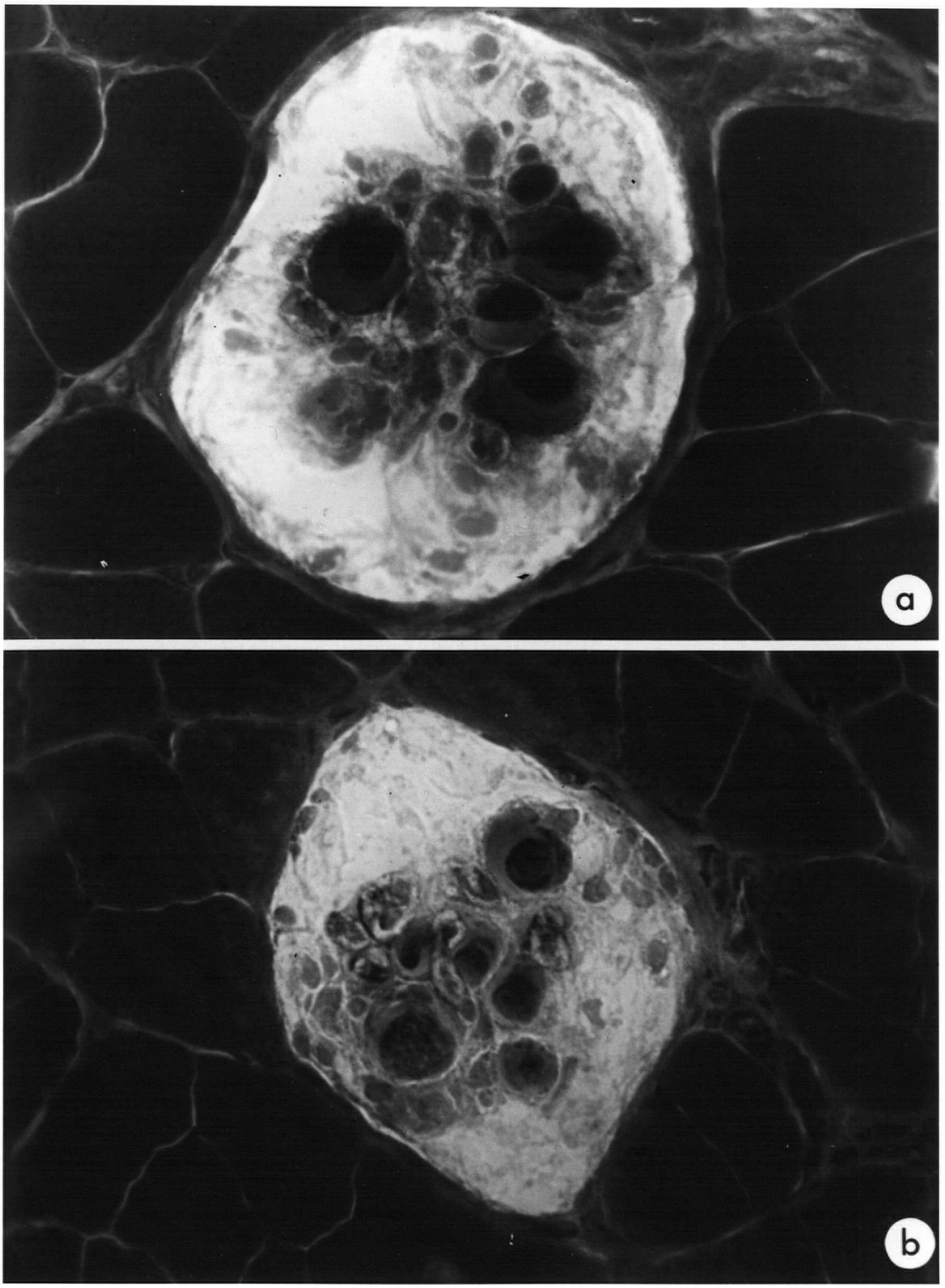
a
b

Figure 13

Transverse paraffin sections through different regions of muscle spindles stained with H and E. X1,000

a. Polar region of a PLD muscle spindle (arrow) containing five intrafusal fibres. Note the adjacent neurovascular trunk.

b. Juxta-equatorial region of an ALD muscle spindle. Note the outer capsule, inner contents of the muscle spindle, and periaxial space (asterisk) compared with those seen in the frozen material.

c. Equatorial region of a PLD muscle spindle. Sensory terminals to two intrafusal fibres are indicated (arrows). Note also the abundance of inner capsule cells and a greatly expanded periaxial space (asterisk).

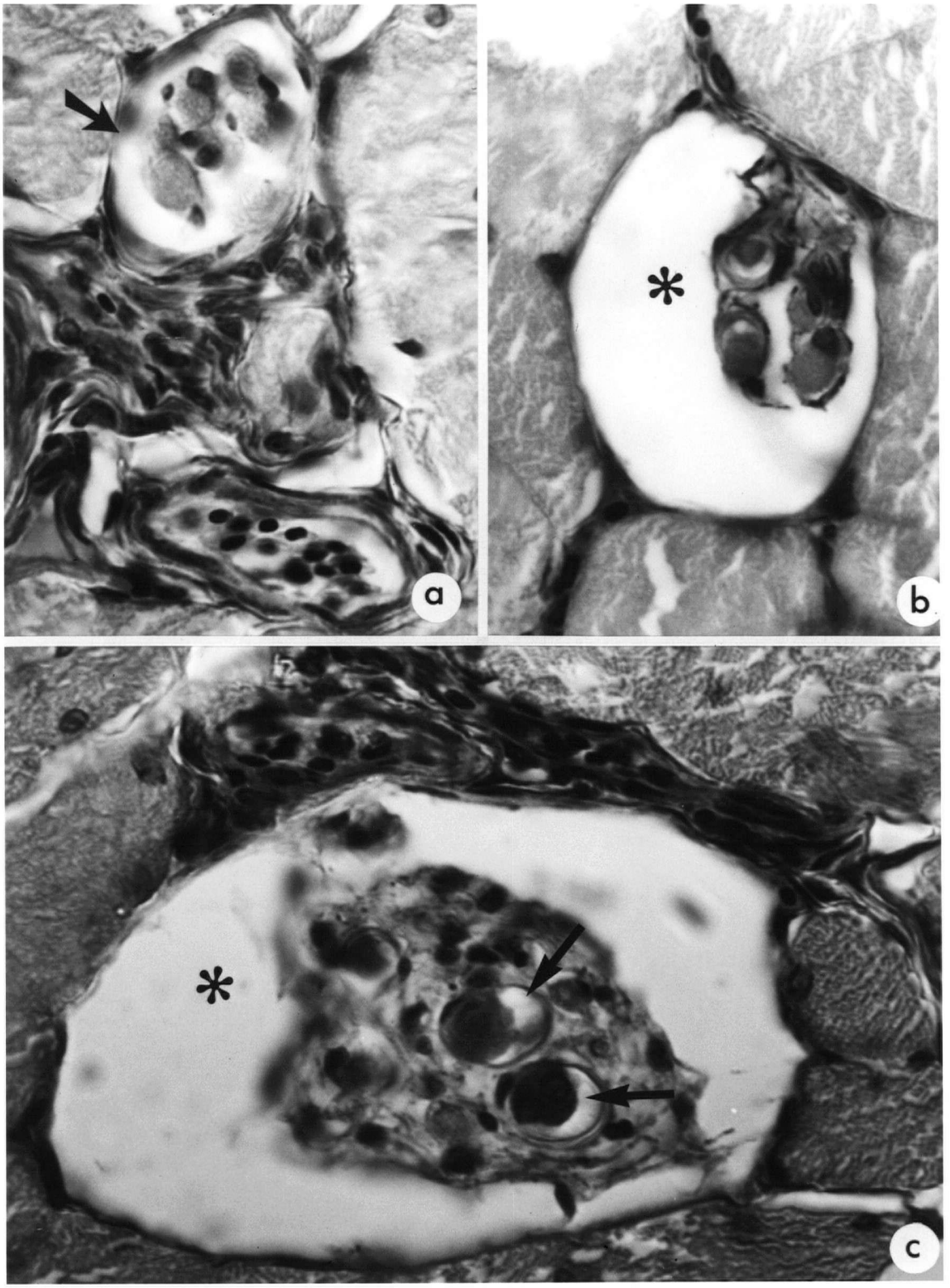
a
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Figure 14

a. Transverse frozen section of a muscular dystrophic PLD. Extrafusal fibres are of irregular size. There is evidence of myofibrillar clumping and connective tissue infiltration between these fibres. A muscle spindle is indicated (arrow). H and E stain. X100

b. Transverse frozen section of a dystrophic PLD. Whereas the intrafusal fibres in this polar muscle spindle appear relatively normal, there is thickening of its outer capsule. The surrounding extrafusal fibres show some signs of degeneration. Gomori trichrome stain. X750

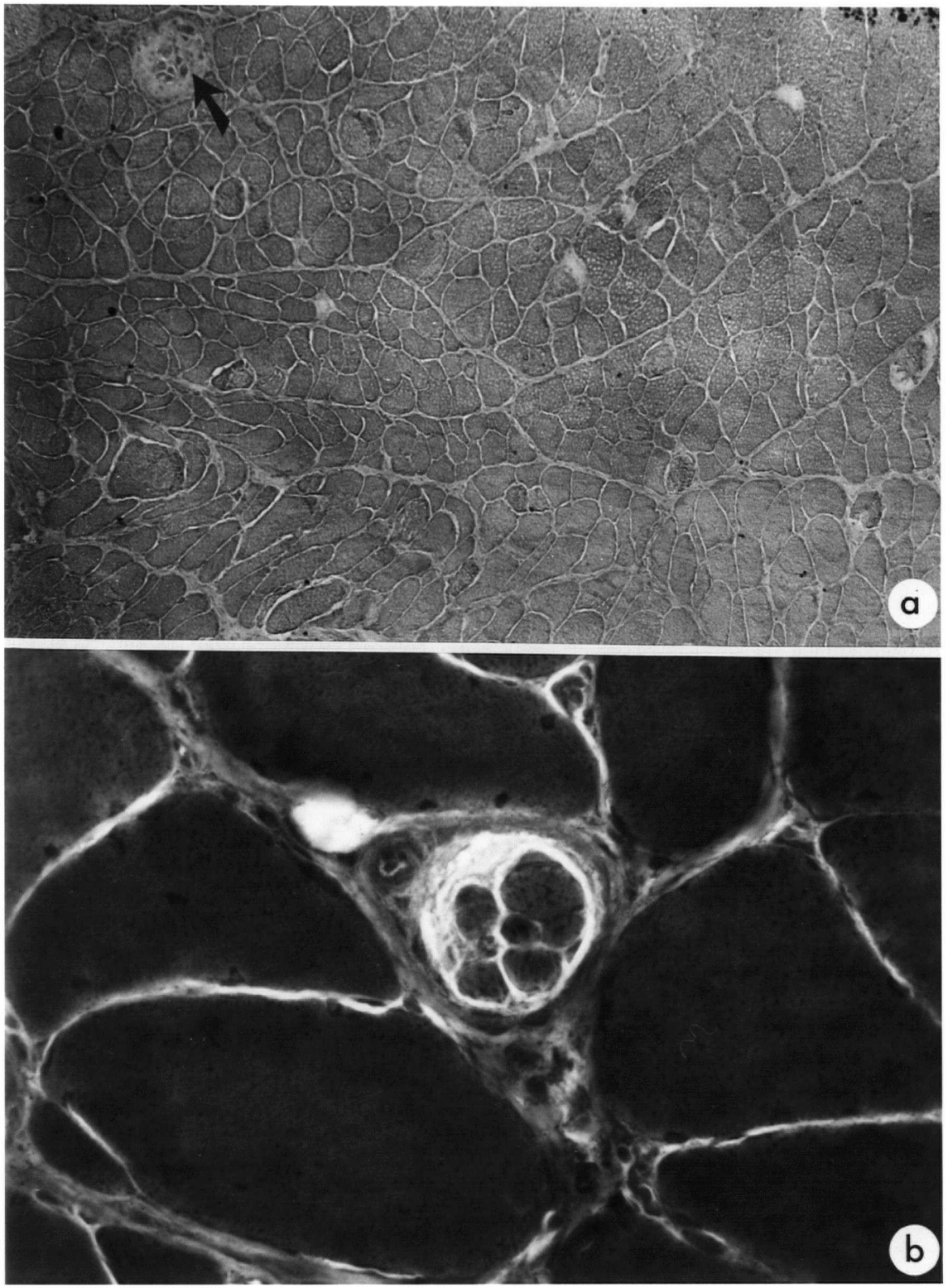
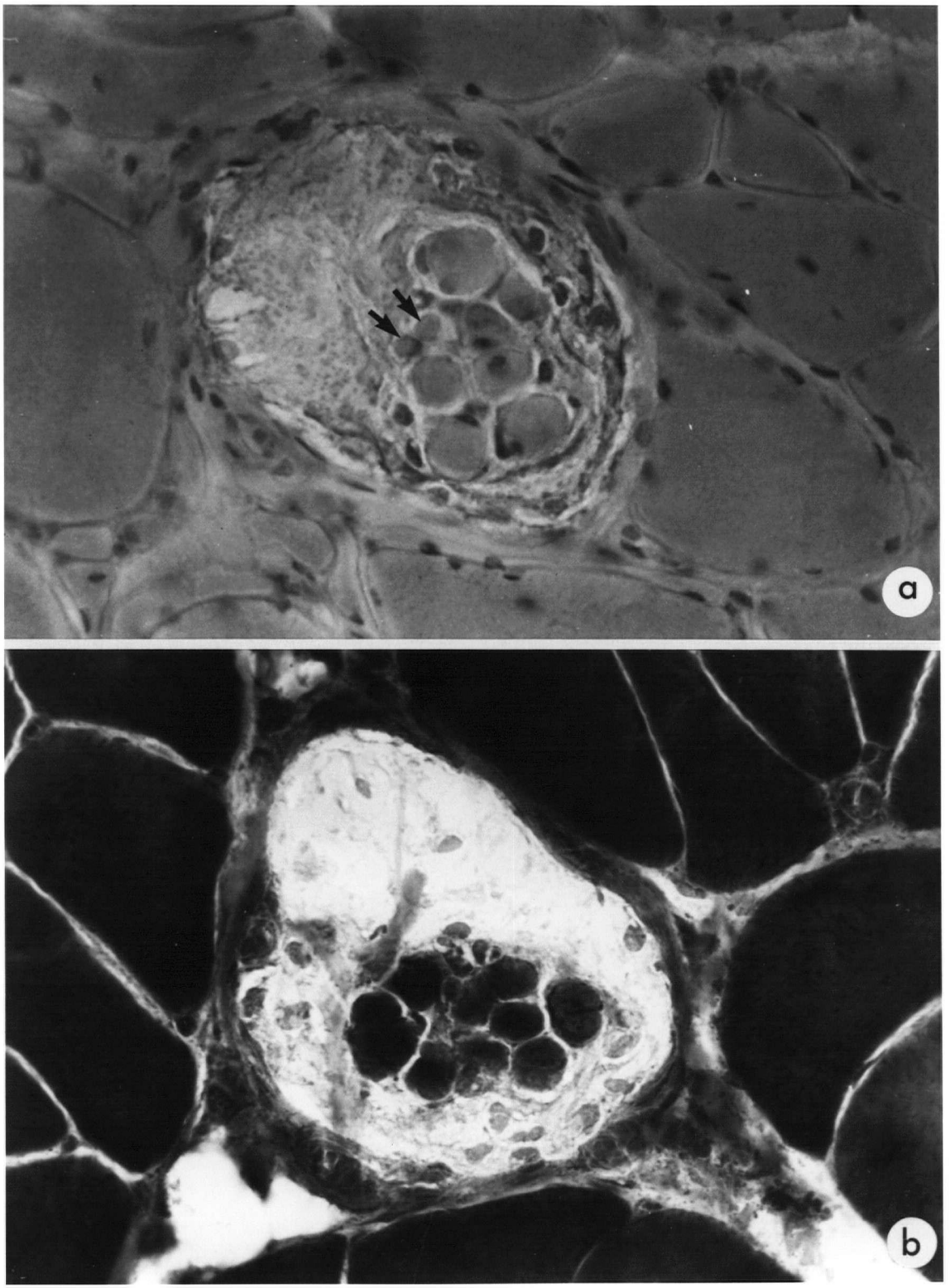

a
b

Figure 15

a. Transverse frozen section of a juxta-equatorial muscle spindle in the dystrophic PLD. The surrounding extrafusal fibres are irregular in size, and many contain centrally-located nuclei. There is evidence of intrafusal fibre splitting (arrows) within the muscle spindle. Some thickening of the outer capsule and infiltration of connective tissue between some extrafusal fibres is seen. H and E stain. X750

b. Transverse frozen section of an equatorial muscle spindle in the dystrophic ALD. Whereas the extrafusal fibres appear unaltered, there is an increase in the number of intrafusal fibres compared with that seen in normal muscles. Eleven intrafusal fibres are seen in this section. Gomori trichrome stain. X750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DISCUSSION

The results of the present study demonstrate that muscle spindles in the slow ALD are different from those in the fast PLD, quantitatively, morphologically and in their relationship with the surrounding extrafusal muscle. When comparing the features of the two muscles it is important to consider their respective functions. Both originate from neural spines of the vertebrae. The ALD arises from the low cervical region and the PLD originates from the thoracic region. Both muscles insert on the upper medial aspect of the humerus. Their principal action would appear to be adduction of the humerus. Functionally, the ALD supports the wing and prevents it from drooping (Vrbova et al, 1978), whereas the PLD demonstrates maximum isometric tension with the wing folded next to the body (Shear, 1978).

It is known that the slow ALD can maintain graded contractions for long periods of time, whereas the fast PLD can change length rapidly, attain high tension, but cannot sustain prolonged contractions (Ginsborg, 1960a; 1960b). Amphibian muscles have been found to contain true slow and fast twitch fibres similar to those in avian species, however the two fibre groups are intermingled within the same muscle (Kuffler and Vaughan Williams, 1953). It is thought that the slow fibres maintain the position developed by the fast fibres and that the two fibre groups act as functional synergists. It is possible that the avian ALD and PLD work in a similar manner to achieve and maintain

optimum wing position, in that the PLD acts as a prime mover in adduction of the wing while the ALD stabilizes the wing in the appropriate position attained by its synergist.

The large disparity between density of muscle spindles in the two muscles reflects their different functions. Slow-twitch mammalian muscles (frequently referred to in the literature as slow muscles), such as the soleus, and those muscles containing a majority of slow-twitch fibres, are known to have significantly higher muscle spindle densities than predominantly fast-twitch muscles (Swett and Eldred, 1960a; Richmond and Abrahams, 1975b). Small postural muscles of the cat neck, such as the intertransversarius, contain up to 500 spindles per gram (Richmond and Abrahams, 1979). Larger neck muscles which maintain overall head position not only have a high muscle spindle density (46 - 106 per gram) but contain the majority in regions of predominantly slow-twitch fibres (Richmond and Abrahams, 1975b). Other mammalian muscles with consistently high spindle densities are those initiating fine movements, such as those controllong distal extremity joints (Chin et al, 1960). Muscles stabilizing important joints, such as masseter and temporalis have also been found to have high spindle densities (Kubota and Masegi, 1977; Lennartsson, 1979). Muscles initiating gross movement, including those operating over large proximal joints, have consistently low spindle indices. It is interesting to note that the human latissimus dorsi, a large muscle which adducts and medially rotates the humerus, has a spindle index of only 1.4 (Voss, 1956).

This study confirms the findings of Adal and Chew Cheng (1980)

and Maier and Eldred (1971) that avian muscles tend to have a higher muscle spindle density than those of mammalian species. The slow ALD contains 130 spindles per gram, a higher density than previously published data on chicken muscle (Maier and Eldred, 1971). As in mammalian slow-twitch muscles the higher spindle index of the ALD probably reflects the necessity of accurate monitoring of small changes in muscle length in order to facilitate correct postural responses.

Although the PLD has a low spindle index compared with its synergist, the highest values in human muscles are no greater (Matthews, 1972), neither are some of the slow-twitch muscles of the cat neck (Richmond and Abrahams, 1975a). Because of the predominant fast-twitch fibre content of the PLD, a lower spindle index than was found in this study would logically be expected, however the importance of the muscle as a functional synergist of the ALD may account for the relatively high values obtained.

ALD muscle spindles were normally located in clefts between muscle fasciculi and generally avoided the heavier planes of connective tissue. In the PLD, with less connective tissue separating the muscle fasciculi, muscle spindles were located either on the periphery, or surrounded by groups of extrafusal fibres. Large slow-twitch postural muscles of the cat neck contain tendinous inscriptions which effectively divide the muscle fibres into contractile sub-units, each with an independent nerve supply from one or more cervical segments (Richmond and Abrahams, 1975a). A similar situation was observed in the ALD which appeared to be divided, both longitudinally and horizontally, by large

planes of connective tissue. Furthermore, the nerve supply originated from three separate trunks of the median nerve, which entered the muscle at different points along the ventral surface. It is proposed that the ALD functions in a complex manner as a group of inter-related sub-units. The lack of interfascicular perimysium and of connective tissue inscriptions in the PLD is not surprising in view of its rapid tension development following an action potential (Ginsborg, 1960b). The presence of structural sub-units within this muscle would tend to decrease the efficiency of muscle contraction.

Muscle spindles usually follow the overall pattern of muscle innervation, although they are not necessarily located in close proximity to the point of nerve entry into the muscle (Barker, 1974). This appeared to be true for the ALD-PLD sample examined in the present study, particularly in the case of the latter, where a single nerve trunk rapidly divided on entering the distal third of the muscle. Approximately 5o% of the total number of spindles were found in this region. The ALD, on the other hand, contained muscle spindles throughout its length, which is to be expected in view of its function in maintenance of wing position and in monitoring small changes in muscle fibre length.

The external capsule of the ALD muscle spindle appeared thicker than that of the PLD, although at the light microscopic level it was sometimes difficult to distinguish the capsule from the surrounding interfascicular perimysium and connective tissue inscriptions. It can only be surmised that the contents of ALD spindles are more isolated from the surrounding extracellular space than those of the PLD,

particularly in the polar region where the capsule is well-developed (Ovalle, 1976). Bridgman and Eldred (1964) suggested that the outer capsule might function as a pressure sense organ, and that in the expanded equatorial region the spindle contents would be subject to increased pressure during muscle contraction. In view of the more intimate relationship apparent between PLD muscle spindles and the surrounding extrafusal fibres than in the ALD, it is suggested that muscle contraction would produce a greater amount of lateral pressure on the PLD spindle contents than on those of the ALD.

There is some evidence that slow-twitch mammalian muscles have longer muscle spindles than fast-twitch muscles (Swett and Eldred, 1960b). In the present study, muscle spindles of the slow ALD were about 20% shorter than those of the fast PLD, however several contributing factors may account for this difference. Monofibril spindles accounted for 11% of the total number in the ALD. Their mean length rarely exceded 50% of the mean spindle length, whereas the majority of spindles with long extracapsular portions were found in the PLD. In addition, the ALD underwent a variable amount of contracture during dissection and isolation, and was frozen at a smaller percentage of its true length than the PLD. A disparity in muscle contracture was reported by Maier and Eldred (1971) when measuring muscle spindle lengths in two avian hip muscles, one fixed in flexion and the other in extension. The extended muscle had 80% longer spindles than the muscle fixed in flexion, and it was suggested that this fact had contributed to the results.

Other than the presence of short monofibril spindles in the ALD sample, the main difference between spindle lengths in the two muscles was seen in muscle spindles containing large-diameter intrafusal fibres. Such fibres frequently had an extracapsular course of 1mm or more at each pole, and were found in spindles containing four or more intrafusal fibres, of which the majority were in the PLD. Unlike the findings of Maier and Eldred (1971), considerable variability in spindle length was seen in both the ALD and PLD sample.

Inner capsule cells were consistently present in the encapsulated portions of both ALD and PLD muscle spindles. In addition, they appeared to increase in number with the development of the periaxial space, particularly in the PLD. Ovalle (1976) noted that ALD and PLD intrafusal fibres were completely surrounded by cytoplasmic processes of inner capsule cells but no difference in their arrangement in the two muscles was noted. In attempting to find reasons for the larger number of inner capsule cells in PLD spindles, the work of Cooper and Gladden (1974) on elastic fibre distribution and function becomes significant. They found that nuclear bag fibres were surrounded by more elastic fibres than were nuclear chain fibres, and that in addition, inner capsule cells were associated with elastic fibres. They suggested that these elastic fibres functioned by compressing intrafusal fibres and their sensory terminals during stretch, resulting in distortion and depolarization of sensory nerve endings. In the present study, large-diameter intrafusal fibres were more frequently seen in the PLD spindles, and it is suggested that the dynamic response to intrafusal fibre stretch

might be enhanced by the interplay between these components.

At this time there is no histochemical confirmation of specific intrafusal fibre types in the PLD, although two kinds of intrafusal fibres have been shown to occur in the ALD (Ovalle, 1978). There is no comparative quantitative data on intrafusal fibre types in ALD and PLD muscle spindles, although studies on other avian muscles have reported either one or three types of fibres.

The results of the present study suggest the existence of two intrafusal fibre populations in the ALD and three in the PLD. If intrafusal fibre characteristics reflect those of surrounding extrafusal fibres, the response of the muscle spindles would reflect muscle function. The ALD, a slow, tonic postural muscle with a contractile mechanism suitable for prolonged, graded contractions, would seem to require an innervation with non-adaptive properties as found on mammalian nuclear chain fibres (Matthews, 1972). Conversely, the PLD, a phasic muscle with twitch contractions would need an innervation with a dynamic component to stretch, as found supplying mammalian nuclear bag fibres (Matthews, 1972). Whereas it is not suggested that fibre size alone could determine intrafusal fibre characteristics, the dynamic component of PLD muscle spindles would be found in the upper half of the intrafusal fibre diameter range. It is possible that some variation in intrafusal fibre type may be influenced not only by the sensory and motor innervation, but by the presence or absence of a fusimotor grape-innervated component such as found in frog muscle spindles (Brown, 1971). Chin (quoted by Barker, 1974) found a collateral grape innervation to ALD

and both a grape and plate fusimotor innervation to PLD muscle spindles.

There is a lack of information on the significance of numbers of intrafusal fibres per muscle spindle, however two studies on the cat have indicated a lower number in postural, slow-twitch muscles than in fast-twitch muscles (Swett and Eldred, 1960b; Richmond and Abrahams, 1975a). In the present study the slow ALD contained 40% fewer intrafusal fibres than the fast PLD and it would be of interest to ascertain whether there is any functional significance in these results. One notable observation in Richmond and Abrahams' (1975a) study is the reduced number of nuclear bag fibres in the slow-twitch neck muscles, when compared with those of the hindlimb. Possibly, the spindles in the slow-twitch muscles have less need for a dynamic component to stretch than those located in the fast-twitch muscles.

An interesting feature of the ALD is the monofibril muscle spindle, reminiscent of those found in the snake and lizard (Proske,1973; Pallot and Taberner, 1973). They have also been reported to occur in the PLD (Chin, quoted by Barker, 1974). Two functional kinds of monofibril spindle are present in the snake, one with a tonic response and the other with a phasic response to stretch or stimulation (Fukami, 1970). On morphological grounds, however, only one type of avian monofibril spindle was identified in the present study. Each spindle in the ALD monofibril sample had a well-developed external capsule, distinct periaxial space and a maximum of one nucleus per intrafusal fibre cross-section. From the published descriptions of two types of snake spindles, the avian monofibril unit would seem to contain features similar to both.

Extrafusal fibre diameter measurements taken from embryonic ALD and PLD muscles up to sexual maturity confirm the results of the present study, in that the slow ALD has larger fibres than the fast PLD. Embryonic ALD fibres, which develop and function earlier than those of the PLD, were found by Gordon et al (1974) to be 100% larger than embryonic PLD fibres, and at age 29 days were 60% larger (Shear and Goldspink, 1973). A similar trend was found by Ashurst and Vrbova (1979) in muscles of 35 day old birds.

Hereditary muscular dystrophy in chickens is specifically expressed in fast-twitch, focally-innervated muscles such as the pectoral and the PLD, whereas slow, multiply-innervated muscles, such as the ALD, are spared disease phenotypes (Cosmos et al, 1979a). These findings were confirmed in the present study, and in addition some morphological changes were noted in muscle spindles, particularly those in the PLD.

The dystrophic PLD showed extrafusal fibre changes similar to those described by Harris and Slater (1980), which are most likely secondary accompaniments of the disease. These include increased variation in fibre diameter, increase in number of myo-nuclei which tended towards central location, fibrosis and connective tissue infiltration. Myofibrillar clumping, a common ultrastructural feature that can also be seen with the light microscope, occured randomly within areas of the muscle belly where fibre degeneration was present. The amount of pathological change varied considerably within the muscle, with areas of normal fibre size adjacent to those showing all the secondary signs

mentioned above.

Hypertrophy of the muscle spindle capsule is a common sequela of neuromuscular disease, and is readily visible with the light microscope (Cazzato and Walton, 1968). Varying amounts of capsular thickening were seen in the dystrophic PLD, within regions of muscle abnormality. It is probable that intrinsic connective tissue proliferation is taking place in the capsule rather than deposition of collagenous material in adjacent structures, because capsular changes were similar whether the spindle was located in interfascicular perimysium or surrounded by muscle fibres. Functional changes in the muscle spindle with hypertrophied capsules is likely to be limited. The sensitivity of primary and secondary innervation could theoretically be modified as a result of increased cushioning of the intrafusal fibres from lateral pressure provided by contracting muscle, however other degenerative processes taking place within the muscle would make verification difficult. Oedematous swelling of the periaxial space, reported by Cazzato and Walton (1968) to occur in limb girdle dystrophy, was not seen in this sample. Pathological changes in PLD intrafusal fibres were difficult to evaluate because of the presence of proliferating collagenous material within the periaxial space. This tended to obscure the outline of the fibres and innervation. Fibre atrophy could not be identified with certainty, however several examples of intrafusal fibre splitting were seen.

In the dystrophic ALD, the mean number of intrafusal fibres increased by 40% over the normal, partly accounted for by the lack of

monofibril spindles. However some change of muscle function is suggested as the reason for this increase. The dystrophic PLD shows an abnormal calcium storage during the last week of embryogenesis, around the time that twitch characteristics are beginning to develop (Cosmos et al, 1979b), while the ALD is already functioning to hold the foetal wing next to the body (Vrbova et al, 1978). By 15 days of age the PLD can only attain 50% of its maximum twitch tension, and it is proposed that the ALD, in the process of compensating for the loss of power in its developing synergist, assumes part of its function. The increase in ALD weight compared with the normal sample is probably a result of two factors: firstly, the two week age difference between normal and dystrophic animals examined, and secondly, compensatory hypertrophy of the muscle. Modification of dystrophic ALD muscle spindles during development might occur if the muscle were forced to alter its function. It is suggested that the ALD not only maintains wing position, but provides part of the initiating force of wing adduction in the absence of normal tension development in the PLD.

The limitations of this study are realized, both regarding the small size of the normal and dystrophic samples. There is need for a further series of experiments, particularly on the histochemistry and fine structure of intrafusal fibres in muscle spindles of the normal PLD. In addition, dystrophic ALD and PLD muscles from animals of various ages could be studied to more accurately assess changes in muscle spindle morphology.

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