Stages in the development of cat muscle spindles

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SUMMARY

The structure of developing spindles has been examined in cat peroneal muscles by light and electron microscopy, beginning at the 34- to 38-day foetal stage.

By this stage α motoneurons have formed end-plates on primary myotubes. Secondary extrafusal myotubes then develop beneath the basal lamina of primary myotubes, and are innervated by motor axons early in their assembly. First-series secondary myotubes separate from primary myotubes prior to the development of subsequent series. The assembly of extrafusal fibres is completed by birth.

Intrafusal fibres assemble in a similar manner. At the 34- to 38-day foetal stage developing spindles consist of a single primary myotube containing a small accumulation of myonuclei beneath the terminals of the la afferent axon. Simple motor nerve terminals also innervate this myotube, which will ultimately become the bag2 fibre of the mature spindle. Secondary intrafusal myotubes then assemble beneath the basal lamina of the primary bag2 myotube, in the order presumptive bag1, long-chain, intermediate-chain and typical-chain fibres. Their assembly begins at the equator, beneath the sensory terminals, and spreads to the poles. The bag1 and long-chain myotubes separate from the bag2 in the spindle pole prior to the development of the other chain fibres. The assembly of intrafusal fibres is completed by birth. The periaxial space begins to develop in the first postnatal week.

The development of tandem spindles containing b2c units is described. The role of sensory and motor innervation in the assembly and differentiation of mammalian intrafusal fibres is discussed.

INTRODUCTION

The first study of muscle-spindle development was made by Sutton (1915), who traced the growth pattern of nerve terminals in the extrinsic eye muscles of the pig. It was not until the investigations of Tello (1917, 1922), mainly in chick, and those of Cuajunco in pig (1927) and man (1940), that the development of all the spindle’s components was recorded.

Most of the subsequent research into spindle development was confined to mammals (see reviews by Smith & Ovalle, 1972; Werner, 1972; Barker, 1974), and particularly to those species where foetal material was readily accessible and easily dated or where development is completed postnatally. The emphasis of the early light-microscopic studies was on the temporal sequence of development of the various spindle components. The first electron-microscopic studies (Landon, 1972; Milburn, 1973) were carried out in rat at a time when knowledge of adult spindle structure had progressed significantly (see Barker, 1974). Their emphasis
therefore lay in resolving the mode of assembly of the various types of intrafusal fibre and the morphogenetic factors controlling this assembly (see Zelená, 1976). Although these first electron-microscopic studies, and a more recent one in mouse (Kozeka & Ontell, 1981) revealed close parallels between the development of extra- and intrafusal fibres, subsequent experimental investigations in rat indicated that the morphogenetic factors operating within the developing spindle are more complex and less well defined than those in the developing extrafusal fascicles (Zelená & Soukup, 1973, 1974; Kronnie, Donselaar, Soukup & Zelená, 1982).

Among the various laboratory animal species, cat has been the traditional choice for intensive physiological and histological study of the mammalian muscle spindle. Whilst the description of muscle-spindle development in other species gave some insight into this process in cat, several characteristic features of adult cat spindles were not explained by the results of these studies. A particular concern was the way in which tandem spindles arise in the muscle primordium, especially those in muscles of the hindlimb (Banks, Barker & Stacey, 1979, 1982; Kucera, 1982a) and neck (Richmond & Abrahams, 1975; Bakker & Richmond, 1981), in which some encapsulations (βc units) exclude the bag1 fibre. In addition this study was of obvious relevance to the wider field of muscle re-innervation and regeneration. Investigations into spindle regeneration following damage to the nerve supply (Brown & Butler, 1976; Barker & Boddy, 1980), or to the muscle itself (Zelená & Sabotková, 1971; Milburn, 1976; Rogers & Carlson, 1981; Barker, Diwan & Milburn, 1982; Rogers, 1982) arose in recognition of the importance of this research for the understanding of neuromuscular disease and for the development of surgical techniques in reconstructive surgery. Clearly the potential of the muscle spindle to regenerate fully following nerve or muscle injury can only be appreciated in the complete knowledge of how it assembles during development.

The preliminary results of part of this study have been reported elsewhere (Barker & Milburn, 1982).

**MATERIALS AND METHODS**

*Assessment of foetal age*

Foetal cats were obtained by the removal of pregnant uteri from adult laboratory females under aseptic conditions. All of the adults recovered successfully from the operation.

One foetal cat (51-days foetal stage) was the sole survivor of a spontaneously aborted litter. The newborn, 1- and 7-day postnatal kittens were members of four separate litters from different mothers.

Excised uteri were placed on a bed of crushed ice while the foetuses were removed. Following dissection of the peroneal muscles (brevis and tertius), the foetal cats were aged on the basis of average crown–rump length (disregarding
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Table 1. Number of litters, specimens and their approximate ages

<table>
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<th>No. litters</th>
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<tr>
<td>34–38 df</td>
<td>1</td>
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<td>38–41 df</td>
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Abbreviations:– df: days foetal; dpn: days postnatal; C–R: crown-rump.

any obviously small or unusual animals), and their external characteristics, in accordance with the data of Evans & Sack (1973). Postnatal cats were aged from the time of birth.

Table 1 gives details of the number of specimens used and their approximate ages.

Excision of muscles

In all but the youngest litter, peroneal muscles were dissected from the lower hindlimb, stretched onto thin card and immersed in cold buffered fixative. Where necessary, the muscles were cut into small pieces (approx. 5×2 mm) after 10 min of initial fixation. All tissue was removed from the card and placed in fresh fixative after 30 min.

In the case of the youngest foetuses (34–36 days), where ossification of the lower hindlimb bones had not started (Boyd, 1971), lower hindlimbs were fixed in toto and the peroneal muscles identified later in transverse sections.

Peroneus brevis of one newborn kitten was fixed and embedded whole for skip-serial sectioning.

Methods for electron microscopy

Muscle tissue was fixed for a total of 2 h at 4°C in Karnovsky (1965) fixative, as modified by M. Saito (personal communication), as follows:

Solution A: 2 g paraformaldehyde in 40 ml distilled water, heated and dissolved with 2–6 drops of 1N NaOH, shaking continuously.

Solution B: 10 ml 25 % glutaraldehyde with 50 ml of 0·2 M-sodium cacodylate buffer, pH 7·3.

The two solutions are freshly prepared, cooled to 4°C and mixed together just before use.

The tissue was then postfixed for 2 h at 4°C in the following solution: 25 ml 4 % osmium tetroxide in 25 ml distilled water, with 50 ml 0·2 M-sodium cacodylate buffer. The tissue was dehydrated in a series of alcohols (50 %, 70 %, 95 %,
Methods for the examination of muscle spindles

All muscles were sectioned on a Reichart OMU3 ultramicrotome using glass knives. Thick (approx. 1 µm) transverse sections, stained with toluidine blue in 1% borax, were scanned with a light microscope for muscle spindles. Once located, spindles were sectioned serially in alternate thick sections (covering a length of 5–20 µm) for light microscopy and thin sections for electron microscopy. In addition two spindles were examined in longitudinal sections following re-orientation of the block.

Thin sections were stained with uranyl acetate followed by lead citrate (Reynolds, 1963) and subsequently examined and photographed with an AEI801 or Philips 400T electron microscope. Thick sections stained with toluidine blue were photographed with a Microflex UFX camera mounted on a Nikon Biological Optiphot microscope.

Of the 34 spindles examined in detail, 16 were reconstructed from pole to pole (2 in longitudinal section in one pole), 10 at the equator only and 8 at the equator and in one pole only. These counts include five tandem spindles in which the bag₁ fibre is excluded from the additional (b₂c) spindle unit, three of which were sectioned through both spindle units, one at the b₂c equator only and one at the equator and poles of the b₂c unit.

In addition, the entire spindle population of one newborn kitten peroneus brevis muscle was serially thick sectioned. Every fifteenth section was stained with toluidine blue and examined with the light microscope. 31 spindles, including 12 tandem spindles, were reconstructed from this one muscle.
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RESULTS

34- to 38-day foetal stage

Extrajeral muscle

Peroneal muscles of the mid-term foetus consist of a mass of primary myotubes arranged in groups of three to six (Fig. 1). The myotubes vary in diameter along their length. Each myotube contains large focal accumulations of glycogen and is surrounded by a rudimentary, patchy basal lamina, that is absent from the
surfaces of closely apposed myotubes. Fibroblasts and myoblasts occupy the spaces between primary myotubes (Fig. 2).

A second type of myotube is occasionally seen among the muscle fascicles. These newly formed secondary myotubes are thinner, shorter and contain fewer myofibrils than the primary myotubes with which they are closely associated (Figs 1B & 2). The association between a nascent secondary myotube and a primary myotube is complex and elaborate and includes the interlocking of extensions of their sarcoplasm at the interface. A common basal lamina encloses the coupled myotubes and some myoblasts (Fig. 2).

Intramuscular nerve trunks and branches have penetrated deep into the muscle primordium by this stage of development (Fig. 1). Bundles of naked axons are surrounded by Schwann cells, processes of which enclose groups of axons in the main nerve trunks. There is no myelin. Intramuscular nerve trunks are small, consisting of up to 30 axons of varying diameters enclosed by a single layer of Schwann cells.

Extramuscular neuromuscular junctions are seen among the myotube groups, forming innervation bands in the muscle. Motor end-plates vary in form; some have clearly defined sole-plates, whereas others lack any postjunctional specialization of the sarcoplasm (Fig. 2). Basal lamina is interposed between terminal and myotube. Nerve terminals contain both clear and dense-core vesicles and innervate nascent secondary myotubes as well as primary myotubes. Single terminals can also cross-innervate two closely associated primary myotubes.

**Muscle spindles**

Two particular features distinguish developing intrafusal myotubes from extramuscular myotubes at this stage of development; firstly, the presence of sensory nerve terminals of the primary Ia axon beneath the basal lamina of the single

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Fig. 2. Low-power electron micrograph of a transverse section of intrafusal and extramuscular myotubes: 34- to 38-day foetus. This section was cut mid-way between sections (B) and (C) of Fig. 1. Spindle 2 (Sp2) contains a single intrafusal myotube with an equatorial bag of nuclei (n). Overlapping sensory nerve terminals (snt) lie beneath the rudimentary basal lamina of the intrafusal myotube. A thin capsule of fibroblast-like cells (cap) isolates the intrafusal myotube from spindle 1 (Sp1) and a neighbouring extramuscular myotube (efmt), that receives motor nerve terminals (mnt). Basal lamina runs between the nerve terminals and the extramuscular myotube. A nascent myotube (arrow) is associated with the extramuscular myotube in this region. Scale bar = 2 \( \mu m \).

Fig. 3. High-power electron micrograph of a transverse section of motor nerve terminals innervating an intramuscular myotube: 34- to 38-day foetus. These terminals were located 70 \( \mu m \) from the equatorial region of spindle 2 which is illustrated in Fig. 2, and between sections (C) and (D) of Fig. 1. They contain both clear and dense-core (arrows) vesicles. Basal lamina occupies the synaptic cleft. Note the thickening of the postjunctional sarcolemma. A Schwann cell (Sc) covers the terminals. Scale bar = 0.5 \( \mu m \).
Development of cat muscle spindles

Figs 2–3
primary intrafusal myotube of the muscle spindle (Fig. 2); and secondly, a small accumulation of nuclei and an increase in the diameter of the intrafusal myotube in the sensory region (Fig. 1A,D & Fig. 2). Beyond this region the single intrafusal myotube is indistinguishable from neighbouring extrafusal myotubes (Fig. 1C).

Sensory nerve terminals innervate only a fraction of the length of the intrafusal myotube (c.100–200 μm), the poles of which extend for at least a further 800 μm. The terminals vary in form. Some take an almost spiral course around the myotube; others form simple contacts, often overlapping with neighbouring terminals (Fig. 2). The flocculent axoplasm contains microfilaments, microtubules, mitochondria and a few clear and dense-cored vesicles. Junctional specializations of the axolemma and sarcolemma, and of the adjacent axolemmata of overlapping terminals are common. Coated vesicle formation is frequently seen at the junctional sarcolemma.

A thin sheath of fibroblasts and Schwann cells encircles the single intrafusal myotube of the developing spindle in the sensory region, isolating it from neighbouring myotubes (Fig. 2). There is no periaxial space.

Developing spindles are not distributed throughout the muscle primordium but are grouped around the intramuscular nerve trunks, with intrafusal myotubes of separate spindles often lying adjacent to one another (Fig. 1). In addition, the sensory region of the intrafusal myotube often lies in the motor innervation zone of the neighbouring extrafusal myotubes (Fig. 2).

Four of the five spindles examined at this stage consisted of single intrafusal myotubes with associated myoblasts. In one spindle, the developing axial bundle contained a nascent secondary myotube associated with the primary intrafusal myotube in the sensory region (Spindle 1, Fig. 1B).

In addition to sensory nerve terminals, the intrafusal myotubes of the three spindles examined in detail also received motor nerve terminals at this early stage of development. Four of the five terminals observed were positioned close
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Fig. 4
to, or overlapped with, the sensory region (Spindle 1, Fig. 1B). These simple motor terminals contain clear and dense-cored vesicles (Fig. 3), are covered by Schwann-cell extensions, and are separated from the primary intrafusal myotube by well-developed basal lamina. No evidence of sole-plate formation was seen, although the postjunctional sarcolemma shows focal thickening (Fig. 3). The intrafusal myotube of one spindle received terminals from a second motor axon some 400 μm from the end of the sensory region.

38- to 41-day foetal stage

Extrafusal muscle

By this stage peroneal muscles are composed of distinctive groups of extrafusal myotubes. Each group includes a large primary myotube and a smaller first-series secondary myotube, which may be closely associated with the primary one, or separated from it as a result of the ingrowth of basal lamina at the interface (Fig. 4). Where this separation is well-advanced, the assembly of the next series of secondary myotubes is indicated by the presence of short nascent myotubes in addition to myoblasts around the walls of the primary myotube (Fig. 4). The separation of the first-series secondary myotube before the assembly of subsequent series around the walls of the primary myotube presents two distinct profiles in the extrafusal fascicles at the completion of this stage of development; firstly, large primary myotubes with one or more satellite nascent myotubes contained within its basal lamina, and secondly, small, separate secondary myotubes with individual basal-lamina sheaths, which lack satellite myotubes and rapidly mature into muscle fibres. These two profiles are strikingly similar to the ‘muscle clusters’ and ‘independent fibres’ described in growing rat hindlimb muscle by Ontell & Dunn (1978).

Figs 5, 6. Low-power electron micrographs of transverse sections through a developing spindle: 38- to 41-day foetus. In the equatorial region (Fig. 5) a distinct capsule encloses the tightly interlocked axial bundle which is composed of a large primary nuclear-bag myotube (Pnb), a thinner, immature secondary nuclear-bag myotube (Snb), a nascent nuclear-chain myotube (nc) and a myoblast (mb), all enclosed in a common basal lamina. Sensory nerve terminals (arrows) are confined to the outer surface of the axial bundle. In the extracapsular polar region (Fig. 6) myofibrils are less dense in the secondary nuclear-bag myotube (Snb) than in the primary nuclear-bag myotube (Pnb). The nascent nuclear-chain myotube seen at the equator (nc in Fig. 5) does not extend into the polar region. Its place in the axial bundle is occupied by myoblasts (mb). A simple motor nerve terminal (arrow) innervates the primary nuclear-bag myotube. Scale bars = 2 μm.

Fig. 7. High-power electron micrograph of the motor nerve terminal illustrated in Fig. 6. The terminal is covered by a Schwann cell (Sc) and is packed with clear vesicles. Note the absence of sole-plate sarcoplasm from the primary nuclear-bag myotube (Pnb). Basal lamina occupies the synaptic cleft. The large, lower, preterminal axon formed terminals on the same primary myotube in subsequent sections. Scale bar = 0.5 μm.
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Fig. 8. Low-power electron micrograph of a transverse section through the equatorial region of a developing spindle: 38 to 41-day foetus. Sensory nerve terminals (arrows) innervate the outer surface of the axial bundle which is composed of a thick primary nuclear-bag myotube (Pnb), a thinner secondary nuclear-bag myotube (Shb), two nascent nuclear-chain myotubes (nc) and a myoblast (mb), all contained in a common basal lamina. Note the close association between the sarcoplasmic extensions of the nascent nuclear-bag myotubes and the nuclei of the primary nuclear-bag myotube. The capsule (cap) is multilayered. Scale bar = 2 μm.
The development of neuromuscular junctions on extrafusal myotubes is widespread by the end of this stage of development, when motor nerve terminals are seen to contact primary, first-series secondary (Fig. 4) and nascent
myotubes of muscle clusters. The neuromuscular junctions of primary myotubes have obvious sole-plates (Fig. 4), although postjunctional folds are rarely seen.

Muscle spindles

The equatorial regions of developing muscle spindles are clearly visible with the light microscope in transverse sections of peroneal muscles at this stage of development.

The spindle now contains two presumptive nuclear-bag (nb) myotubes. The primary nb myotube is thicker, longer and contains more equatorial nuclei than its small partner (Figs 5, 8) which has developed beneath the same basal lamina. In some spindles the smaller secondary nb myotube is still immature. It contains few myofibrils (Figs 5, 6) and has pseudopodial-like extensions of its sarcoplasm that invade the invaginated surface of the primary nb myotube. The common basal lamina also encloses myoblasts (Figs 5, 6) and, in the sensory region, at least one nascent myotube wedged between the nb myotubes. In other spindles the secondary nb myotube has lost its pseudopodial attachments with the primary myotube, and nascent myotubes now extend well beyond the sensory region. Pseudopodial extensions of nascent presumptive nuclear-chain (nc) myotubes more frequently invade the sarcoplasm of the primary nb myotube than the secondary one, and are often associated with its central nuclei (Fig. 8). The formation of coated vesicles is seen at the apposed sarcolemmatae of nascent and primary intrafusal myotubes.

The muscle spindle thus consists of at least three closely interlocked myotubes with sensory nerve terminals on their outer surfaces in the region enclosed by the

Fig. 10. Low-power electron micrograph through the motor end-plate region of peroneal muscle: 41- to 43-day foetus. Two groups of developing extrafusal muscle fibres are present. Each group consists of a large primary myotube (P, P'), with nascent secondary myotubes (mt) and myoblasts (mb) enclosed in a common basal lamina, and a first-series secondary muscle fibre (S1, S1'), containing well-developed myofibrils. These first-series secondary fibres are separated from the primary myotubes and enclosed in their own basal lamina. In addition, each group contains immature thin secondary fibres (S2, S2'), which are closely associated with the primary myotubes. Myoblasts (mb) are associated with both primary and secondary fibres. Sole-plate sarcoplasm (arrows) is prominent at the motor end-plates of primary myotubes and first-series secondary fibres (S1). Scale bar = 1 μm.

Figs 11, 12. High-power electron micrographs of transverse sections through motor nerve terminals innervating extrafusal myotubes: 41- to 43-day foetus. In Fig. 11 a primary myotube (P) exhibits a distinct sole-plate and early secondary synaptic-cleft formation (arrows). Some of the motor nerve terminals (mnt) are, in addition, closely associated with a nearby secondary fibre (S1) and in some regions of this association basal lamina is absent from the junctional cleft. In Fig. 12 a nascent secondary myotube (mt) and primary myotube (P) share a motor nerve terminal (mnt). In addition each of the myotubes receives its own nerve terminals (arrows). Scale bars = 0.5 μm.
thin sheath. There is no periaxial space (Figs 5, 8). Sensory nerve terminals, many of the overlapping type, are commonly shared by neighbouring myotubes, including nascent myotubes (Fig. 8). Both nb myotubes have M-lines in their myofibrils in all regions.

Beyond the limits of the sheath, the profile of the intrafusal myotubes may alter as the secondary nb myotube, accompanied by a long nascent nc myotube (when present), begins to separate from the primary nb myotube by the development of basal lamina at the interface, and the invasion of fibroblasts. At the extreme poles of the developing spindle the nb myotubes lie apart, each enclosed by individual basal lamina, and are indistinguishable from extrafusal myotubes. Where the spindle inserts into developing tendon, the primary nb myotube may extend into the tendon some 200 μm beyond the end of the secondary nb myotube and extrafusal myotubes (Fig. 9). Myoblasts often lie beneath the rudimentary basal lamina of the primary nb myotube in this region.

Motor nerve terminals now innervate the newly formed nc myotubes of the axial bundle. Postjunctional folds and sole-plates are absent from all intrafusal neuromuscular junctions (Fig. 7).

41- to 43-day foetal stage

Extrafusal muscle

The assembly, maturation and separation of secondary extrafusal myotubes is well advanced by this stage of development (Fig. 10). Each extrafusal muscle group is composed of the following: a muscle cluster, consisting of a thick primary myotube with nascent myotubes and myoblasts applied to its walls, all of which are enclosed in a common basal lamina; nearby, one or two thin immature secondary muscle fibres, each completely or partially enclosed by individual basal lamina (S2, S2', Fig. 10) and lastly a thicker, distinctly separate first-series secondary muscle fibre with well-developed myofibrils (S1, S1', Fig. 10).

Neuromuscular junctions are well established on primary myotubes and the first-series secondary fibres (Fig. 10), where they exhibit prominent sole-plates.
and early synaptic-cleft formation (Fig. 11). Motor nerve terminals also innervate the thin secondary muscle fibres and nascent myotubes of the muscle clusters. Individual terminals may innervate more than one myotube (Figs 11, 12).

Muscle spindles

Intrafusal myotubes have also increased in number by the development of one or more additional nc myotubes in association with the more mature myotubes. Pseudopodial extensions of nascent nc myotubes often preferentially associate with the primary nb myotube, although this is not always the case (Fig. 13). Nascent myotubes are largely confined to the intracapsular region of the

Fig. 15. (A–F). Light micrographs of skip-serial transverse sections of a developing spindle: 41- to 43-day foetus. The distances between sections (A) to (F) are respectively 125, 275, 165, 170 and 370 µm. The intrafusal myotubes are labelled as follows:- primary nuclear-bag myotube (1); secondary nuclear-bag myotube (2); long-chain myotube (3); nuclear-chain myotube (4); nascent nuclear-chain myotubes (mt, mt'). The distinct capsule encloses two separate sensory regions. In the main sensory region (C and D) all of the intrafusal myotubes received sensory nerve terminals, including the nascent nuclear-chain myotube (mt) which is exclusive to this main sensory region. In the second, shorter, sensory region (E), a separate nascent nuclear-chain myotube (mt') is associated with the primary myotube. Sensory nerve terminals were distributed exclusively to the primary nuclear-bag myotube in this region. Note how the secondary nuclear-bag myotube has started to separate from the rest of the axial bundle at the equator (arrows in C), as well as at the spindle poles (A, B and E, F). The long-chain myotube was as long as the secondary nuclear-bag myotube and lay apart from the rest of the axial bundle only in the extracapsular polar regions (A and F). 1 µm-thick Araldite sections stained with toluidine blue. Scale bar = 5 µm.

Fig. 16. (A–F). Light micrographs of skip-serial transverse sections through a developing tandem spindle: 41- to 43-day foetus. The distances between the sections (A) to (F) are respectively 100, 195, 75, 75 and 40 µm. The intrafusal myotubes are labelled as follows:- primary nuclear-bag myotube (1); secondary nuclear-bag myotube (2); long-chain myotube (3); nascent nuclear-chain myotube of main encapsulation (mt); nascent nuclear-chain myotube of smaller encapsulation (mt'). At the spindle pole (A), the secondary nuclear-bag and long-chain myotubes lie apart from the primary nuclear-bag myotube. Between (B) and (C) the closely interlocked axial bundle received sensory nerve terminals, and myotubes 1 and 2 displayed nuclear bags. A nascent nuclear-chain myotube (mt) is present. At the spindle’s distal pole (D) myotubes 2 and 3 again dissociate from the primary myotube. Between (D) and (E), myotubes 1 and 3 received motor nerve terminals. At (E) and (F) the primary nuclear-bag myotube is separately encapsulated and receives sensory nerve terminals (snt). A nascent myotube (mt') was confined to this second capsule. Beyond (F) the primary myotube inserted into tendon, after the insertion of myotubes 2 and 3. 1 µm-thick Araldite sections stained with toluidine blue. Scale bar = 5 µm.

Fig. 17. Low-power electron micrograph of a transverse section through the smaller of two encapsulations of a tandem spindle: 41- to 43-day foetus. The secondary nuclear-bag myotube (Snb) of the main encapsulation is excluded from this smaller encapsulation. A distinct capsule (cap) encloses the primary nuclear-bag myotube (Pnb) as well as a nascent nuclear-chain myotube (mt), which is exclusive to this smaller second encapsulation. Sensory nerve terminals (arrows) of unmyelinated axons (ax) encircle the outer surface of the axial bundle. Scale bar = 2 µm.
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Fig. 17 for legend see p. 194
developing spindle (Figs 13, 15) and their assembly begins in the region of the sensory innervation. The closely interlocked myotubes frequently share sensory nerve terminals, which are now located on both their outer and inner surfaces (Fig. 13).

The separation of the secondary nb myotube (and the long nc myotube if it is present) from the rest of the axial bundle is now seen in the intracapsular ‘sleeve’ region of the developing spindle (Fig. 15B,E), as well as in the polar regions (Figs 14, 15A, F). In some spindles the separation has spread through the equator (Fig. 15C).

The capsule is composed of three to five layers of flattened attenuated cells (Fig. 13) that also cover the spindle nerve trunk, the axons of which are still unmyelinated. Collagen fibrils are associated with the outer surface of the interlocked myotubes in the equatorial region. There is no periaxial space.

In one of the spindles examined in detail at this stage of development, a second group of sensory nerve terminals innervated the primary nb myotube some 75 μm from the end of the primary sensory innervation (Fig. 15E). A nascent myotube, exclusive to this second sensory region, was associated with the primary nb myotube (Fig. 15E). Both sensory regions were contained within one capsule (Fig. 15C,D,E), suggesting that the additional group of sensory nerve terminals was probably derived from a Group II afferent axon.

In three spindles examined at this stage, an additional separately ensheathed sensory region lay some 200–300 μm apart from the main one (Fig. 16). The second capsule contained the primary nb myotube and a closely apposed nascent myotube that is exclusive to the additional ensheathed region (Figs 16F, 17). Sensory nerve terminals encircle the outer surface of both myotubes which are additionally enclosed by a thin capsule. The secondary nb myotube (Fig. 17) and long nc myotube when present (Fig. 16E,F) are excluded from the second encapsulation of the developing tandem spindle. In two spindles the primary nb myotube left the second capsule to insert into developing tendon.

Motor nerve terminals innervate all myotubes of the axial bundle, including the intercapsular region of the primary nb myotube of developing tandem spindles.

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Figs 18, 19. Low-power electron micrographs of transverse sections through a muscle spindle: 7-day postnatal. In the juxtaequatorial region (Fig. 18) the bag1 (b1) fibre and one of the long-chain (lc') fibres lie apart from the rest of the axial bundle. Sensory nerve terminals (arrows) are commonly shared between chain fibres (c1–c4). Note the thin periaxial space (pas). In a section through the intracapsular polar region (Fig. 19) taken 300 μm distal to the previous section, the association of the long-chain fibres (lc and lc') with the b1 fibre is accompanied by the partitioning of the capsule. One compartment contains the b1 and both long-chain fibres, and the other the b2 and other chain fibres (c1–c4), one of which (c4) is close to insertion. Both bag fibres bear motor end-plates (arrows). The b2 and chain fibres continued to be separately encapsulated for a further 300 μm where the chain fibres inserted. Scale bar = 3 μm.
Muscle spindles

During the later stages of foetal development, additional nc intrafusal myotubes assemble exclusively in association with the primary nb myotube, now recognizable as the presumptive bag₂ fibre. The separation of the secondary nb and the long nc myotubes from the rest of the axial bundle has spread to the equator of the spindle where they are now recognizable as presumptive bag₁ and long-chain fibres. Terminals of the Ia afferent axon separately encircle the presumptive bag₁ fibre, which is of a similar diameter to the bag₂.

Among the group of nc myotubes associated with the primary bag₂ myotube, the longest and most mature one is often separated from the rest of the group in the intracapsular polar regions while remaining closely associated with the rest at the spindle equator.

Newborn to 7-day postnatal stage

Extrafusal muscle

By birth muscle clusters are no longer distinguishable in the extrafusal fascicles. The majority of extrafusal fibres are contained in individual basallamina sheaths, although there are considerable variations in fibre diameter (Fig. 20). Primary and late-developing secondary fibres are distinguishable only where separation of the foetal muscle cluster is incomplete and basallamina is shared. Postjunctional folds and sole-plates are well developed at neuromuscular junctions and motor nerve terminals are rarely shared between extrafusal fibres.

Fig. 20. (A–H). Light micrographs of skip-serial transverse sections through a tandem spindle: 1-day postnatal. The distances between the sections (A) to (H) are respectively 45, 395, 85, 60, 105, 75 and 70 µm. The tandem spindle consisted of two units with a distance of 690 µm separating the two equatorial regions illustrated in (A) and (F). The axial bundle of the main unit (A and B: see also Fig. 21) is composed of two nuclear-bag fibres (b₁ and b₂) and seven nuclear-chain fibres (c). Note the thin periaxial space. At the termination of the capsule (C) the chain fibres (c) have started to insert and a second population of chain fibres (c') begins in this region. The second unit (D to G) encloses the b₁ fibre and the second population of chain fibres (c'). The b₁ fibre and longer chain fibres of the b₁b₂C unit are excluded from this second unit (D and E). Here sensory nerve terminals are distributed to both the b₁ and chain fibres. The b₂ fibre lacks a bag of nuclei at the equator of the second unit (F). The pole of the b₂C unit (G and H) inserted into an aponeurosis, after the insertion of the excluded b₁ fibre. Motor nerve terminals innervated the b₁ fibre close to the termination of the b₁b₂C capsule (at C). The b₂ and chain fibres of the b₁b₂C and b₂C units received motor nerve terminals in the intracapsular polar regions (between B and C, and C and D respectively). 1 µm-thick Araldite sections, stained with toluidine blue. Scale bar = 10 µm.
Muscle spindles

The number of intrafusal myotubes has increased by the development of nc myotubes exclusively in association with the presumptive bag₂ fibre (Figs 18, 20A,B, 21). In some newborn kitten spindles nascent nc myotubes still closely appose the presumptive bag₂ fibre, with which they may share common sensory nerve terminals, whereas the more mature nc fibres lie apart. In others the group of short nc myotubes all lie apart from the presumptive bag₂ fibre, which, like the presumptive bag₁ and long-chain fibres, lies in a separate compartment of the inner capsule, where it receives terminals from the Ia afferent axon (Figs 18, 21). The group of separated short nc fibres commonly share sensory nerve terminals and basal lamina (Figs 18, 21).

Away from the primary sensory region most of the more mature nc fibres lie separate from the bag₂ cluster, so that the dissociation of the presumptive bag₁ and long-chain fibres becomes less obvious than at earlier stages of development (Fig. 20B). In addition, the increase in girth of the bag₁ and older chain fibres in this region has started to eliminate the distinctive diameter differences between the various fibres that are seen at earlier foetal stages.

Motor nerve terminals innervate all intrafusal fibres in the intracapsular polar regions. Those terminating on the bag₁ fibre at the end of the capsule (Fig. 19) are large and clearly visible with the light microscope as they deform the fibre surface. In addition, the bag₁ and longest chain fibres are often contained in separate compartments of the capsule in this region (Fig. 19). Other bag₁ terminals are smaller and the junctions display sole-plates and postjunctional folds. The motor terminals of bag₂ and nc fibres are smaller than bag₁ terminals. The neuromuscular junctions of nc fibres always display junctional folds.

Both collagen and elastic fibrils are associated with the capsule and axial sheath cells. In the first postnatal week the periaxial space begins to appear (Fig. 18). Nerve axons are still unmyelinated.

Tandem spindles, including b₂c units (Banks et al. 1982) are easily identified

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Figs 21, 22. Low-power electron micrographs of transverse sections through the two units of a tandem spindle: 1-day postnatal. The ultrathin section in Fig. 21 was cut adjacent to the thick section illustrated in Fig. 20(B) and is through the juxta-equatorial region of the b₁b₂c unit. The dissociation of the bag₁ (b₁) fibre from the bag₂ (b₂) and chain (c) fibres is less obvious because of the separation of the chain fibres from the b₁ fibre in this region. Sensory nerve terminals (arrows) are commonly shared between chain fibres. Note the thin periaxial space (pas). The ultrathin section in Fig. 22 was cut adjacent to the thick section illustrated in Fig. 20(E) and is through the juxtaequatorial region of the b₂c unit. The b₁ fibre of the b₁b₂c unit is excluded from this second capsule which contains the b₂ fibre, common to both capsules, and 5 chain fibres (c'), exclusive to this capsule. Most of the chain fibres are closely associated with the b₂ fibre and enclosed in a common basal lamina. Sensory nerve terminals are shared by the b₂ and chain fibres (arrow) as well as by individual chain fibres. Note the thin periaxial space (pas), and myoblasts (mb). Scale bars = 2 μm.
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in peroneal muscles at this stage. In one tandem spindle examined in detail (Figs 20, 21, 22), the two capsules, separated by a distance of only 300 µm, contained two distinct and separate populations of nc fibres which overlapped in the intercapsular region (Fig. 20C). The b2C unit of this tandem spindle contained a nascent nc myotube closely apposed to the presumptive bag2 and nc fibres (Fig. 22). Sensory cross terminals between the bag2 and nc fibres, and between the nc fibres were common. The bag1 fibre of the associated b1b2C unit (Banks et al. 1982) was excluded from the second encapsulation (Figs 20D–G, 22), which inserted into an aponeurosis (Fig. 20H). Motor nerve terminals innervated both groups of intrafusal fibres between the two equatorial regions.

Skip-serial sectioning of a newborn kitten peroneus brevis muscle showed that the 31 spindles present included 12 tandem spindles, of which seven contained b2C units. The average intercapsular distance between the tandem units of the five b1b2C/b1b2C tandem spindles was 350 µm compared with an intercapsular distance of 250 µm between the tandem units of the seven b1b2C/b2C tandem spindles.

General pattern of assembly

The observations of cat peroneal muscle at different stages of development indicate that the pattern of intrafusal-fibre assembly is essentially similar to that of extrafusal fibres, and the two processes are compared in Fig. 23.

By the 34- to 38-day foetal stage motor and sensory nerve axons have grown into the muscle primordium. Terminals of α motoneurons have formed end-plates on primary myotubes and secondary extrafusal myotubes have begun to assemble. A first-series secondary myotube assembles in association with each primary myotube, separating from it to form an innervated independent fibre some 3 days later, when it is a distinctive feature of the muscle fascicles (Fig. 23). Generations of smaller secondary extrafusal myotubes than develop exclusively in association with primary myotubes, from which they subsequently separate.

Fig. 23. Schematic diagrams of transverse sections of developing extrafusal and intrafusal muscle fibres in cat peroneal muscles. In the extrafusal fascicle note how the first-series secondary myotube (stippled) separates from the primary myotube (black), acquiring its own basal lamina (stippled halo), before the assembly of subsequent series of secondary myotubes (white). The thin fusiform cells (hatched) are myoblasts. The diagrams of intrafusal muscle fibres show how myotube assembly begins at the equator and spreads to the poles, in contrast to their maturation and separation, which begins at the poles and spreads to the equator. The column headed 'insertion into tendon' shows the later arrival of a b2C afferent axon as compared with a b1b2C Ia axon; it is shown innervating the primary bag2 myotube of a developing spindle and initiating the subsequent development of a b2C spindle unit of a tandem spindle. Abbreviations: α, alpha motoneuron; b1, bag1 fibre; b2, bag2 fibre; b1b2C, developing b1b2C spindle unit; b2C, developing b2C spindle unit; df, days foetal; ic, intermediate-chain fibre; lc, long-chain fibre; tc, typical chain fibre; Ia, Ia axon.
Motor nerve terminals innervate extrafusal myotubes early in their formation. By birth, growth of the independent secondary fibres has started to eliminate the distinction between the different generations of muscle fibres.

Spindle development has already begun in peroneal muscles of the 34- to 38-day foetus. Each spindle consists of a single primary myotube containing a small central accumulation of myonuclei beneath the terminals of a Ia afferent axon. This primary nuclear-bag myotube will ultimately become the bag₂ fibre of the
mature spindle. Simple motor nerve terminals also innervate the primary intrafusal myotube at this early stage.

These pioneering axons then initiate the sequential generation of secondary intrafusal myotubes in association with the primary nuclear-bag myotube, within a common basal lamina. Their assembly begins beneath the terminals of the sensory nerve axons that encircle the outer surface of the axial bundle, and spreads towards the poles, as in rat (Landon, 1972; Milburn, 1973) and mouse (Kozeka & Ontell, 1981). As development proceeds, successive secondary myotubes are shorter and thinner and contain fewer equatorial nuclei, so that those formed last of all become the shortest and thinnest ‘typical’ (Kucera, 1980a) chain fibres of the mature spindle.

In the 41- to 43-day foetal spindle, the separation of the first-series secondary intrafusal myotube (the presumptive bag₃ fibre) from the rest of the axial bundle has spread towards the equator of the spindle, where it eventually acquires individual terminals from the Ia axon. At the same time, the bulk of the nuclear-chain fibres assemble exclusively in association with the primary myotube, from which they subsequently separate, often as a group. During this equatorial separation sensory cross-terminals between presumptive bag₁ fibres, and bag₂ and chain fibres are lost, the Ia innervation is remodelled and the mature form of the ending is established.

Secondary afferent axons probably arrive at the site of spindle development by contact guidance with the Ia axon (Banks et al. 1982) and were first seen in 41- to 43-day foetal spindles, when the separation of the presumptive bag₁ and long-chain myotubes had begun to spread to the equator (Fig. 23). The first secondary axon to reach any spindle would be likely to terminate in an S₁ position and supply all three fibre types. Any that arrive later terminate in more polar positions where the myotubes are well separated. The selective innervation of presumptive bag₂ and nascent chain fibres is thus more probable, and this may account for the differential distribution of secondary endings to the three types of cat intrafusal fibre (Banks et al. 1982).

The intrafusal fibres of cat thus develop in the order bag₂, bag₁, long chain, intermediate chain and typical chain, and their equatorial position in the mature spindle reflects the sequence of their assembly and maturation. The long-chain fibre is not a feature of every cat spindle; in development it is the first chain fibre to form after the nb fibres. The long chain and bag₁ myotubes then follow a similar pattern and time course in their separation from the rest of the axial bundle. This premature separation is one of the factors that leads to their exclusion from b₂c units of tandem spindles.

These b₂c units arise in development as a consequence of the innervation of a primary nuclear-bag myotube, already engaged in spindle development, by a late-arriving b₂c afferent axon. An additional and separate population of nuclear-chain myotubes then assembles in association with the primary myotube beneath the terminals of the b₂c afferent, forming a b₂c tandem spindle unit (see Fig. 23).
DISCUSSION

Development of extrafusal muscle fibres

During development, mammalian skeletal muscle fibres begin to assemble in the absence of nerve terminals. Peripheral nerves grow into the muscle primordium as the mononucleated myoblasts fuse to form the primary generation of myotubes (Kelly & Zacks, 1969), which extend from tendon to tendon (Ontell & Dunn, 1978). In this study primary myotubes were seen in cat peroneal muscles by the 34- to 38-day foetal stage.

In rat both innervation and muscle electrical or contractile activity are essential for the development of secondary generation myotubes (Harris, 1981a). They form by the fusion of myoblasts and develop in close apposition to the walls of primary myotube, within a common basal lamina (Landon, 1970, 1971; Kelly & Zacks, 1969; Kelly & Schotland, 1972; Ontell & Dunn, 1978; Stickland, 1982). The multicellular profile so produced has been named a 'muscle cluster' (Ontell & Dunn, 1978). Once a certain degree of differentiation is attained, secondary fibres separate from primary ones to become new independent muscle fibres (Kelly & Schotland, 1972; Ontell & Dunn, 1978; Harris, 1981a).

The assembly, maturation and separation of secondary fibres takes longer in cat than in rat, and the generations of secondary myotubes can be separated into different series according to their sequence of development. The premature separation of the first-series secondary myotube noted in this study may also be a feature of developing rat muscle. Kelly & Schotland (1972) described and illustrated independent myotubes in 18- to 20-day foetal rat intercostal muscle. These myotubes differ from the larger primary myotubes by the absence of nascent myotubes from their walls. The authors considered the smaller independent myotubes to be part of the primary population, which they first identified in the 16-day foetus. It seems more likely, particularly in the light of Harris' (1981a) study, that these independent myotubes assemble in association with primary myotubes at the 17-day foetal stage, separating from them by the 18- to 20-day foetal stage.

The concept of muscle histogenesis consisting of the assembly of successive series of myotubes is not without precedent. Wirsen & Larsson (1964) proposed that the various histochemical types of mouse muscle fibres assemble in one of three separate generations of myotubes, which become smaller as development proceeds. Whether or not the first-series secondary myotubes identified in this study are qualitatively different from subsequent series, as they proposed, requires further study.

In a series of experiments on the embryonic growth and innervation of rat diaphragm muscle, Harris (1981a) has shown that primary myotubes develop autonomously but new secondary fibres cannot develop unless there are nerve terminals available. In this study, motor nerve terminals were seen to innervate the short, newly formed myotubes of the muscle cluster, and Ontell (1977) has.
described myoneural junctions on satellite fibres still closely associated with primary fibres in postnatal rat hindlimb muscle. These observations support Harris' (1981a) proposal that innervation is essential for the proper histogenesis of skeletal muscle fibres, rather than proposals which suggest that myotubes are innervated late in their assembly (e.g. Kelly & Schotland, 1972; Rubinstein & Kelly, 1981).

Development of muscle spindles

General pattern of assembly of intrafusal muscle fibres

The first electron-microscopic studies of spindle development in rat (Landon, 1972; Milburn, 1973), and a more recent one in mouse (Kozeka & Ontell, 1981) revealed close parallels between intra- and extrafusal fibre development. In rat, it was proposed that the nuclear-bag fibres develop exclusively in the presence of sensory innervation, in the order 'typical-bag' fibre (bag$_2$), 'intermediate-bag' fibre (bag$_1$) (Milburn, 1973), whereas the chain fibres develop in the first postnatal days, after the arrival of the motor innervation at birth. A similar sequence was observed by Cuajunco in spindles of pig (1927) and man (1940), where the large-diameter fibres were recognized as the most advanced and included in the initial formation of the spindle. In pig, Cuajunco further suggested that medium-sized fibres form from myotubes grouped with the first fibres, and that the small fibres form last of all. In complete contrast to this general pattern of spindle development, the intrafusal fibres of rat tail muscles are reported to assemble in the absence of any sensory innervation, which is first seen in spindles at the end of the first postnatal week (Ovalle, 1976).

From the observation of perinatal kitten spindles, Butler (1979, 1980) proposed that cat intrafusal fibres, unlike rat fibres, develop as two separate groups, one group consisting of the bag$_1$ fibre and the second consisting of the bag$_2$ and chain fibres. These observations led Kucera (1982$b,c$) to suggest that long-chain fibres develop from myotubes associated with the bag$_1$ fibre, whereas all other chain fibres develop in association with the bag$_2$. In this study developing spindles were examined from a wider range of foetal stages than Butler studied, and the results reveal that all secondary intrafusal myotubes (including the bag$_1$ and long chain when present) develop in association with the presumptive bag$_2$ myotube, from which they later separate. Clearly the developmental groups described by Butler represent a late stage in the maturation of the axial bundle, and do not reflect the pattern of assembly of the various intrafusal fibres.

In addition to long-chain fibres, Kucera (1980$a$) has identified two other sub-populations of chain fibres in some cat hindlimb muscle spindles. Intermediate chain fibres are longer than typical chains and often dissociate from the bag$_2$ and typical chains at the spindle equator. The series of nuclear-chain myotubes that are the first to develop after the bag$_1$ or long-chain myotube in cat spindles are longer than subsequent series, and separate from the presumptive bag$_2$ fibre in
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advance of the shorter, younger nuclear-chain myotubes. Because of their greater length and premature separation it seems reasonable to suggest that the first series of nuclear-chain myotubes develop into intermediate chain fibres and the subsequent series, that often separate as a group, form the typical chain fibres of the mature spindle.

The role of innervation in spindle development

Motor innervation. The results of this study have shown that most cat intrafusal myotubes receive motor nerve terminals early in their assembly. This suggests that motor axons arrive at the site of spindle formation soon after the Ia afferent axon, or at the same time, as in man (Mavrinskaya, 1967). The nature of these motor axons and the distribution of their terminals amongst the various cat intrafusal myotubes requires further study. It may be that the motor innervation of foetal cat spindles differs considerably from that of adult spindles, in its form, the distribution of its terminals and even its function. It may play an important role alongside the sensory innervation in the assembly of intrafusal myotubes, and later be remodelled to the adult form, as occurs with the Ia afferent innervation and possibly the motor innervation of extrafusal fibres (Betz, Caldwell & Ribchester, 1979; Harris, 1981b). The concept of a changing motor innervation during development is not without precedent. In cat it has been suggested that when 'optimum matching' occurs between an intrafusal fibre and fusimotor neurones, the fibre may reject earlier connections with other neurones (Arbuthnott et al. 1982).

There is a considerable weight of experimental evidence to suggest that in rat the Ia afferent innervation plays the predominant role in the assembly and differentiation of intrafusal fibres (see review by Zelená, 1976). In rat hindlimb muscle, denervation at birth arrests spindle formation and the denervated muscle becomes devoid of spindles (Zelená, 1957, 1964). In contrast to this, de-efferented newborn rat spindles develop the full complement of differentiated intrafusal fibres (Zelená & Soukup, 1973, 1974), except for deficiencies in the myosin characteristics of nuclear-bag fibres (Kronnie et al. 1982). Newborn rat spindles may have undergone their initial development in the presence of a motor innervation, as in cat and mouse (Kozeka & Ontell, 1981) and its influence may already be exerted by birth. If this is the case, then the conclusions drawn from the results of the experimental studies must be revised.

Evidence of the morphogenetic role of the spindle's motor and sensory innervation during development has been obtained from investigations into the innervation, histochemistry and morphology of individual adult cat intrafusal fibres (e.g. Kucera, 1981a,b). It is unlikely that such indirect evidence on its own will provide insight into the role of sensory and motor nerve axons in spindle morphogenesis, particularly when the form and distribution of their nerve terminals changes as the spindle matures. The particular role of the motor innervation will only be revealed when its form and distribution in the developing spindle is
better understood, and when experimental techniques are devised that permit
the investigation of spindle development in the absence of motor innervation.
These techniques are more likely to be successful in muscle regenerates than in
developing muscle, particularly when applied to spindles that form de novo in
grafted avian muscle (Mackenson-Dean, Hikida & Frangoulakis, 1981).

Sensory innervation. Although the role of the motor innervation in spindle
development is poorly understood, there is little doubt that an afferent innerva-
tion is essential for the increment and maintenance of cat intrafusal fibres (see
Zelená, 1976), and that this dependency decreases with age (Werner, 1973;
Schiaffino & Pierobon Bormioli, 1976). De-afferentation of adult spindles
results in the loss of intrafusal equatorial nuclei and a reduction in the periaxial
space (Tower, 1932; Boyd, 1962; Kucera, 1980b). Similarly, deficiencies in the
Ia afferent re-innervation of regenerating rat nuclear-bag fibres is closely cor-
related with deficiencies in the regeneration of equatorial bags of nuclei (Mil-
burn, 1976; Barker et al. 1982; Rogers, 1982). It seems likely therefore that the
Ia afferent axon stimulates the accumulation of myonuclei beneath its terminals
during development.

The source of the additional intrafusal myonuclei that permit the development
or regeneration of nuclear bags remains obscure. Electron-microscopic studies
of spindle development have failed to produce evidence of mitosis in intrafusal
myotube nuclei. It therefore seems improbable that either mitosis (Marchand &
Eldred, 1969) or amitosis is the source of equatorial nuclei.

In a previous study of rat spindle morphogenesis (Milburn, 1973) it was
proposed that the extent of the equatorial nucleation of intrafusal fibres is a
reflection of the decreasing morphogenetic influence of the Ia afferent axon
during development. Those fibres that are the first to form (nuclear-bag fibres)
thus develop larger accumulations of equatorial nuclei than those forming last of
all (the nuclear-chain fibres), where the influence of the sensory axon merely
retains the myotubal nature of the underlying fibre. This proposal is supported
by the results of this study in cat, where chain fibres, that may display small bags
of nuclei in adult spindles (Kucera, 1982b), were seen to develop after the
nuclear-bag fibres but before the typical chain fibres.

In cat the full development of the equatorial nucleation of nuclear-bag fibres
is a gradual process, which may not be completed until some time after birth
(Maier & Eldred, 1974). With the exception of the primary nuclear-bag myotube
at the time of its first innervation, developing intrafusal myotubes are innervated
communally on their outer surfaces by terminals of the Ia afferent. Variations in
equatorial nucleation may be an expression of differences in the duration of the
dependence of intrafusal myotubes on the Ia afferent axon. Those that form first
of all may be dependent for a longer period than those forming later, although
this does not appear to be the case in rat (Werner, 1973). More probably, the
basic equatorial differences between cat intrafusal fibres are laid down in the
foetus under the influence of the pioneering Ia axon. These differences then
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increase in the young kitten when the Ia afferent innervation has remodelled by
the growth of individual terminals around the bag1 and bag2 fibres.

There is evidence from the examination of adult cat spindles that some secon-
dary afferent axons may exert an influence on the underlying intrafusal nuclei
during development. Banks et al. (1982) have described accumulations of
myonuclei beneath the terminals of an S1 afferent in the bag2 and bag1 fibres of
a reconstructed spindle. In this study the first secondary afferent axons to arrive
at the developing spindle appear to promote the formation of myotubes beneath
their terminals. These two observations suggest that some Group II afferents can
induce the development of intrafusal myotubes, although this does not appear
to be a prerequisite for the development of the various types of intrafusal fibre,
since normal cat spindles can lack secondary sensory innervation (Banks et al.
1982).

Development of tandem spindles

In the early stages of cat development, the presumptive bag2 fibre of some
spindles has two separately encapsulated sensory regions. By the first postnatal
week each capsule contains separate populations of intrafusal myotubes, which
overlap but do not fuse in the intercapsular region. The smaller capsule contains
bag2 and chain fibres only, and is clearly distinguishable from the additional
capsules of other tandem spindles which contain the full complement of
intrafusal muscle fibres. For these reasons the additional encapsulated sensory
regions of foetal presumptive bag2 fibres are defined as a stage in the develop-
ment of b2c units of tandem spindles. The postnatal growth of the intercapsular
region of these tandem spindles requires further study.

These b2c spindle units are not exclusively associated with the tendinous inser-
tions or aponeuroses of cat hindlimb muscles (Kucera, 1982a). Their development
in other areas of the muscle is probably linked to the pattern of maturation of the
presumptive bag1 and long-chain fibres, which separate from the bag2 myotube
in the polar regions at the early foetal stages. Innervation of the presumptive bag1
and long-chain fibres by b2c afferent axons must be suppressed by factors possibly
related to their early maturation, leaving the separated bag2 myotube open to
innervation. The extensive motor innervation of extrafusal myotubes by the 41-
to 43-day foetal stage probably prevents their innervation by b2c afferent axons.

Apart from deficiencies in the number and types of intrafusal fibres, there is
a general reduction in the equatorial nucleation of the intrafusal fibres of b2c
tandem units. The reasons for these deficiencies are obscure. They may be
related to the relative maturity of the presumptive bag2 myotube when innervated
by the b2c afferent compared with its condition when innervated by the b1b2c Ia
afferent axon. In support of this, experimental studies of spindle development
and regeneration indicate that presumptive intrafusal fibres are receptive to the
influence of sensory axons for a limited period (Zelená, 1964; Zelená & Sabot-
ková, 1971; Barker et al. 1982). In addition, the morphogenetic influence of b2c
afferent axons may be tempered by the surrounding tendon, or the neural influence itself may differ from that exerted by normal Ia afferent axons.

Attempts to isolate b2C afferents by monitoring the stretch responses of cat spindle afferents have met with limited success (Richmond & Abrahams, 1979; Banks, Ellaway & Scott, 1980). Banks et al. (1982) have classified the afferent axons of b2C spindle units as primary rather than secondary on the basis of several features, including the nucleation of the underlying intrafusal fibres and the occasional presence of a secondary afferent in these capsules. However Kucera (1982a) failed to observe any secondary afferents in b2C units of cat tenuissimus muscle. The results of this study indicate that b2C afferent axons innervate intrafusal myotubes at the same foetal stage as S1 afferents, which may also induce intrafusal myotube development. Whether b2C afferents are a separate group of Ia axons that induce the development of a functionally significant spindle unit, or are no more than late-arriving Ia axons or aberrant secondary axons that produce an accidental spindle variant requires further investigation.

A recent histochemical evaluation of cat tenuissimus spindles (Kucera, 1982a), revealed that a very small number of single spindles contain only one bag fibre, of either the bag2 or bag1 types.

Whilst their infrequent occurrence suggests they are a product of aberrant spindle development (Kucera, 1982a), their existence, along with b2C spindle units shows clearly that the pattern of intrafusal-fibre assembly is not immutable.

The majority of cat tandem-spindle units contain the full complement of intrafusal fibres (e.g. Kucera, 1982a) forming b1b2C/b1b2C tandems (Banks et al. 1982). These tandem linkages were identified in newborn kitten hindlimb muscle. It seems likely that they arise from the synchronized multiple innervation of a single primary myotube by two or more separately derived Ia axons. Each encapsulated sensory region would then recruit its own population of secondary intrafusal myotubes that remain exclusive to each capsule (Barker & Ip, 1961; Richmond & Abrahams, 1975; Kucera, 1982a).

Kozeka & Ontell (1981) have described the structure of developing tandem spindles in 19-day foetal mouse hindlimb muscle, in which both presumptive nuclear-bag myotubes are continuous from one capsule to the next. These observations led Kucera (1982a) to suggest that tandem spindles arise because of an asynchrony in the time of arrival of the separate Ia axons. It is doubtful that this proposal can be extended to cat, since descriptions of the structure of conventional cat tandem spindles indicate that only the bag2 fibre is continuous from one unit to another.

General implications of the pattern of spindle development

The results of this study provide a firm morphological base for further investigations into reported abnormalities in the afferent response of immature cat spindles (Skoglund, 1960a,b) and will thereby contribute to a better understanding of spindle function.
The early innervation of both extrafusal and intrafusal myotubes by terminals of motoneurons is of particular relevance to investigations into the development of metabolic differences between muscle fibres and to those concerned with the factors that lead to the elimination of extrafusal fibre polyneural innervation (see Bennett, 1983). In addition, the presence of a motor innervation early in cat spindle morphogenesis requires the re-investigation of spindle development in other species, particularly in rat, where this has been the subject of experimental study. Investigations into the regeneration of mammalian spindles have so far been confined to rat. It is therefore essential that spindle development is fully documented in this species.

Knowledge of the pattern of spindle development in cat, coupled with the results of the extensive investigations into spindle function in this species provides a basis for studies into spindle regeneration in higher mammals. This is a line of research within the general field of muscle regeneration that has been neglected despite its clinical importance for the development of techniques for muscle repair and for the understanding of neuromuscular disease.

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