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SYMPOSIUM ON MUSCLE SPINDLES

(UNIVERSITY OF DURHAM, 4–6 APRIL 1974)

Introduction

The Symposium was attended by 61 participants including 23 from overseas. It began with an introductory lecture by the organizer, Professor D. Barker, on Thursday afternoon, 4 April, and ended at lunchtime on Saturday, 6 April. The introductory lecture was addressed mainly to those members of the Anatomical Society working in other fields, who attended the Symposium out of interest. With them in mind the first papers dealt with human spindles, and this session was followed by tea and demonstrations. The following day was spent on amphibian and reptilian spindles in the morning and mammalian spindles in the afternoon. The final session on Saturday morning also concerned mammalian spindles, particularly aspects of their motor innervation. Before the start of the non-mammalian papers on Friday, some time was spent considering the development of rat spindles. In the absence of Dr Zelená, owing to circumstances beyond her control, Prof. Barker drew attention to the main points in her demonstration, which she would otherwise have communicated as a paper (see abstract D. 5; hereafter numbers in brackets refer to abstracts of Symposium papers or demonstrations).

One of the main points recognized during the Symposium was that there are two types of nuclear-bag muscle fibre in mammalian limb spindles (see D. 4, 12). They differ with respect to length, diameter, histochemical profile, ultrastructure, and development. In cat the longer and thicker ('typical') type has medium to high levels of activity with phosphorylase (P'ase), succinic dehydrogenase (SDH), and actomyosin ATPase after alkali pre-incubation (Alk ATPase); it also has a medium level of glycogen. The shorter and thinner ('intermediate') type, on the other hand, has low levels of P'ase and Alk ATPase activity, medium to high SDH activity, and a low level of glycogen. Ultrastructurally these differences are matched juxta-equatorially by the longer and thicker type having little interfibrillar sarcoplasm; a poorly developed sarcotubular system; small, infrequent mitochondria; and a double M line (two thin lines); whereas the shorter and thinner type has a moderate amount of interfibrillary sarcoplasm; a well-developed sarcotubular system; larger and more numerous mitochondria; and a single prominent M line. A paper on human spindles (4) showed that there is also a difference in the distribution of elastic fibres around the two types of bag fibre. It was agreed that a final decision on naming the two types should await the correlation of histochemical and ultrastructural characteristics in one and the same spindle, preferably in a number of different species.

The highlights of the session on non-mammalian spindles were the film on intrafusal muscle-fibre contraction in long-capsule snake spindles (9) and the precise

correlation shown between film and electron microscopy of the sensory region of short-capsule snake spindles (10). Reports of work on frog spindles showed that much more was now known about the ultrastructure (7) and motor innervation (D. 3) of their muscle fibres, through there was still surprisingly little information about the equatorial structure of the small type.

Several papers on mammalian spindles dealt with fusimotor innervation by β-axons (19, 20, 21, 24). Electrophysiological evidence was presented demonstrating the presence of β axons in a variety of cat hindlimb muscles. These axons usually exert a dynamic action on primary endings, but a few appear to produce a genuinely static action (21). These findings are what might be expected in the light of the histological evidence concerning the distribution of p_1 plates in cat hindlimb muscles. A paper on intrafusal glycogen depletion elicited by β axons in cat spindles (24) showed that stimulation of dynamic β axons usually results in a selective depletion of the intermediate type of bag fibre. On the other hand, experiments in which the identity of individual muscle fibres activated by dynamic γ axon stimulation was ascertained by fluorescent and electron microscopy (23), apparently indicate a selective involvement of the typical type of bag fibre. It is now generally accepted that static γ axons usually activate both bag and chain fibres. Two papers (20, 23) presented evidence to this effect; one of them (23) specified that both types of bag fibre are involved. It was agreed that firm conclusions about the types of bag fibre activated by dynamic fusimotor axons would have to await the correlation of their histochemical and ultrastructural characteristics.

Experiments concerned with various aspects of the afferent discharge from spindles were reported. The dynamic phase of the receptor potential in cat primary endings was shown to consist of an early component related to the initial burst followed by a late component associated with the dynamic phase of the impulse discharge (15). Comparison of instantaneous frequency plots and receptor potentials suggests that some accommodation takes place at the impulse generating site. Spindles situated in different parts of muscle were shown to respond quite differently to similar muscle stretches owing to lack of mechanical homogeneity of extrafusal structures at the spindle sites (25). The response of primary endings to brief periods of repetitive stimulation of single dynamic γ axons was shown to be enhanced by muscle stretch; such potentiation was not observed after stimulation of γ static axons (18). An increase of the K^+ concentration was shown to alter the discharge and response to stretch of rat primary endings (16). Various effects were described which might explain some of the muscular symptoms resulting from prolonged muscle contraction. In a paper dealing with the controversial matter of the action of the sympathetic nervous system on spindles, evidence was presented showing that naturally released catecholamines may directly induce an increase of the frequency of firing of rat primary endings (17).

The long-debated problem of the inhibition of fusimotor activity by muscle stretch was reconsidered (26). Hunt's observation on the inhibitory effect of stretch in decerebrate cat was confirmed. It was further shown that the γ motoneurons inhibited by stretch were inhibited by recurrent collaterals from α motor axons. In spinal cats no inhibition was observed, but after *DOPA* injection some fusimotor motoneurons, presumably static, were also inhibited by muscle stretch.

As the participants took their leave, the general impression was that the Sympo-

sium had been a very friendly and constructive meeting. A cold Arctic mist enveloped Durham throughout and ensured that heads kept cool, thought clearly, and saw and heard little other than muscle spindles.

D. BARKER
Y. LAPORTE

COMMUNICATIONS

1. Sensory innervation of primate muscle spindles. By KATHLEEN P. FOX, T. H. KOEZE and M. SWASH (introduced by D. BARKER). *Section of Neurological Sciences, The London Hospital, London*

Physiological experiment has revealed the presence of a group of sensory endings in mammalian muscle spindles whose response to applied stretch is intermediate between that of typical primary and secondary sensory endings. In histological preparations of normal primate spindles the complexity of the pattern of innervation is such that it is difficult to distinguish secondary endings from motor endings. We have therefore studied the sensory innervation in de-efferented baboon muscle, using a silver block impregnation method.

Fifty two spindles were studied in three animals. Each spindle contained one primary ending in its equatorial region, usually consisting of a complex network of fine, unmyelinated axons and terminals. These endings were supplied by 55 group Ia afferent nerve fibres whose mean axon diameter was 5·53 μm (s.d. ± 0·695). Seven spindles received only primary sensory innervation. Eighty one secondary endings were studied (mean afferent axon diameter 3·3 μm: s.d. ± 0·88): 57 were located in the S1 position (mean afferent axon diameter 3·58 μm: s.d. ± 0·803), 20 in S2 (mean afferent axon diameter 2·68 μm: s.d. ± 0·67), 3 in S3 (mean afferent axon diameter 2·03 μm), and one in S4. In 36 spindle poles there was a single secondary ending in the S1 position and in 21 there was an additional S2 secondary ending. The mean afferent axon diameter for the former group was 3·37 μm (s.d. ± 0·77), and for the latter 3·95 μm (s.d. ± 0·75). For these differences in mean afferent axon diameter $P < 0{\cdot}01$. Many S1 secondary endings consisted of a spiral network similar in form to that of typical primary endings and most of these seemed to be intimately mixed with the terminals of the adjacent primary endings.

These findings were considered in relation to the physiological distinction between primary and secondary sensory endings in the baboon.

2. Human muscle spindles: fine filaments in Ia sensory endings. By W. R. KENNEDY and H. DE F. WEBSTER (introduced by D. BARKER). *Department of Neurology, University of Minnesota, Minneapolis, U.S.A.* (Fig. 1)

In our continued studies to correlate the structure and function of human muscle spindles we examined the three-dimensional structure of the normal Ia sensory ending. Fine filaments, 75 Å in diameter, and similar to the actin-like microfilaments of cultured nerve growth cones, were found in all levels of one serially sectioned spindle, and in all selected sections of spindles from muscle biopsies of seven other normal subjects. The filaments occurred in two general arrangements. The first consisted of large central aggregates of densely packed parallel filaments, which followed the course of the ending as it encircled the intrafusal muscle fibre. The aggregates occupied up to 40% of the cross sectional area of some endings, and were partially encircled by mitochondria. Individual filaments appeared to react with heavy mero-myosin. The transversely sectioned nuclear-bag intrafusal muscle fibre in Fig. 1A receives three sensory endings. Each ending contains one or two large aggregates of fine filaments oriented longitudinally (left) or transversely (right and bottom) to the plane of the section (× 10800). The second arrangement was a network of fine filaments in the periphery of the sensory ending profile. These approached, and often appeared to merge with, the plasma membrane. They often intervened between the plasma membrane and the filament aggregates (Fig. 1B × 56500).

The aggregates of fine filaments in sensory endings resemble the sheath microfilaments

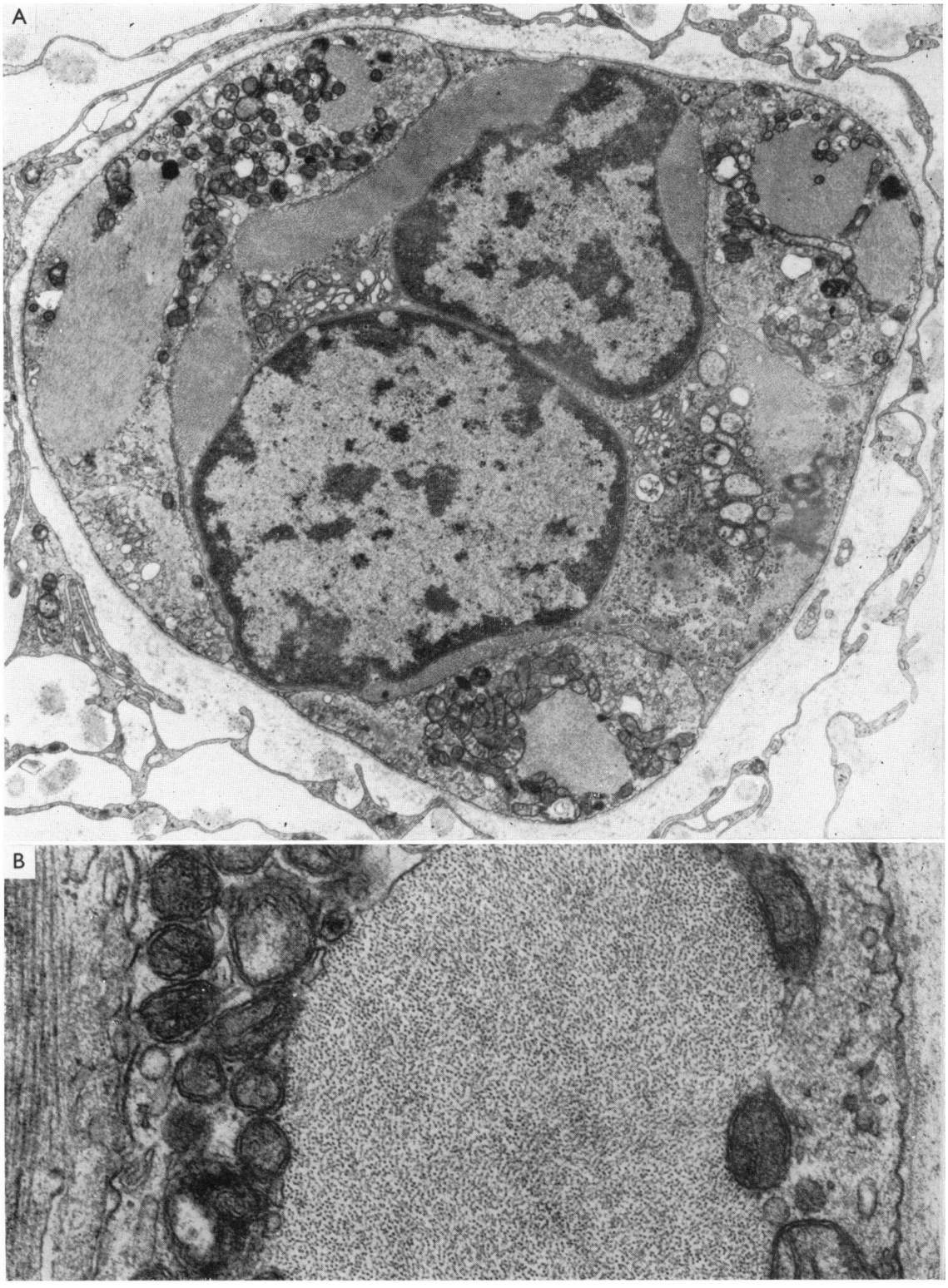

Fig. 1

described in cultured glial cells, fibroblasts, and macrophages. Network filaments are similar to the microfilaments in nerve growth cones and Pacinian corpuscles. Microfilaments are thought to be involved in cell movement. The contributions of the two arrangements of fine filaments in altering the shape of the sensory endings during changes in spindle length are currently being investigated.

3. Discharge properties of human intercostal muscle spindles studied *in vitro*. By J. NEWSOM DAVIS (introduced by D. BARKER). *Department of Neurology, Royal Free Hospital, London*

An *in vitro* preparation has been developed, based on the biopsied intercostal muscle, which allows a combined study of the discharge properties and structure of individual human muscle spindles (Newsom Davis, *J. Physiol.* **231**, 1973). The muscle spindles are placed in modified Tyrode solution, usually maintained at 31–35 °C. The spindle is identified under the dissecting microscope and the muscle fascicle in which it lies is dissected free, or in some cases the spindle itself is isolated. One end of the fascicle is attached to a tension transducer and the other to an electromagnetic vibrator driven by a wave form generator. The imposed ramp or sinusoidal length changes are recorded by a transducer in parallel. The afferent nerve is brought up into a layer of liquid paraffin for monophasic recording. At the end of the experiment, the spindle is fixed and processed for microscopy.

The slope of the frequency-length plot has ranged from 4 to 47 impulses/s/5 % extension, implying a position sensitivity *in vivo* of about 3–30 impulses/s/mm, if one assumes an *in vivo* intercostal muscle length of 3 cm. No clear difference in position sensitivity has been apparent for primary and secondary endings. The dynamic index has proved to be an approximately linear function of the velocity of stretch over the range of velocities available (maximum 10 mm/s). Secondary endings have a dynamic index of less than 15 impulses/sec at a stretch velocity of 3 mm/s and have not been driven by vibration frequencies above 50 Hz. Primary endings can be regularly driven by vibration frequencies up to 200 Hz and in some instances at frequencies higher than this if relatively large amplitudes are used. But it has not yet proved possible to drive human intercostal spindles at the high frequencies to which cat intercostal spindles respond, suggesting a possible species difference.

4. Elastic fibres in human muscle spindles. By MARGARET H. GLADDEN (introduced by D. BARKER). *Institute of Physiology, Glasgow University* (Fig. 2)

Intrafusal muscle fibres are well endowed with elastic fibres, unlike extrafusal fibres. The extracapsular parts of human lumbrical spindles have thick, longitudinally-running elastic fibres, which branch at the beginning of the capsule. These branches follow the outer capsule, or cross the periaxial space with the intrafusal bundle.

Since motor endings occur mainly outside the periaxal space, tensions occurring during intrafusal contraction must be transmitted across this space to the primary sensory terminals by the myoplasm and sarcolemma (including plasma membrane and collagen) of intrafusal fibres and by elastic fibres. The myoplasmic area (solid lines in Fig. 2) of intrafusal fibres was approximately estimated in serial transverse sections by measuring the fibre areas and subtracting the areas of any nuclei present (solid areas). The periaxial space began at section 0 and the equatorial region occupied sections 55–85. As the myoplasmic area of one nuclear-bag fibre decreased (NB_1, sections 30–60) elastic fibres surrounding it became prominent (indicated by crosses). Elastic fibres were only prominent around the other bag fibres (NB_2, NB_3) close to the equatorial region, just before a marked fall in their myoplasmic areas (sections 50–65). In the same region the elastic fibre numbers in the inner capsule rose (lowest graph). The total myoplasmic area of the nuclear-chain fibres (NC) changed little. These results suggest that elastic fibres compensate mechanically for decreases in intrafusal fibre myoplasm. In cat spindles, in which elastic fibres surround all nuclear-bag fibres (Gladden, *J. Physiol.* **227**, 1972), elastic fibres may transmit relatively more tension than in human spindles.

Twenty one human spindle poles had one nuclear-bag fibre like NB_1; all other nuclear-bag fibres resembled NB_2 and NB_3. Thus two kinds of nuclear-bag fibre were distinguished, differing

in their equatorial structure. Direct observations of spindles have shown that nuclear-chain fibres are operated by static γ axons whereas nuclear-bag fibres may be operated by either static or dynamic γ axons, in which case it appears that different nuclear-bag fibres are involved (Bessou & Pagès, *C. r. hebd. Séanc. Sci. Paris*, **277**, 1973; Boyd, Gladden, McWilliam & Ward, *J. Physiol*, **230**, 1973). The differing equatorial structure of nuclear-bag fibres described above may account for this differing functional behaviour.

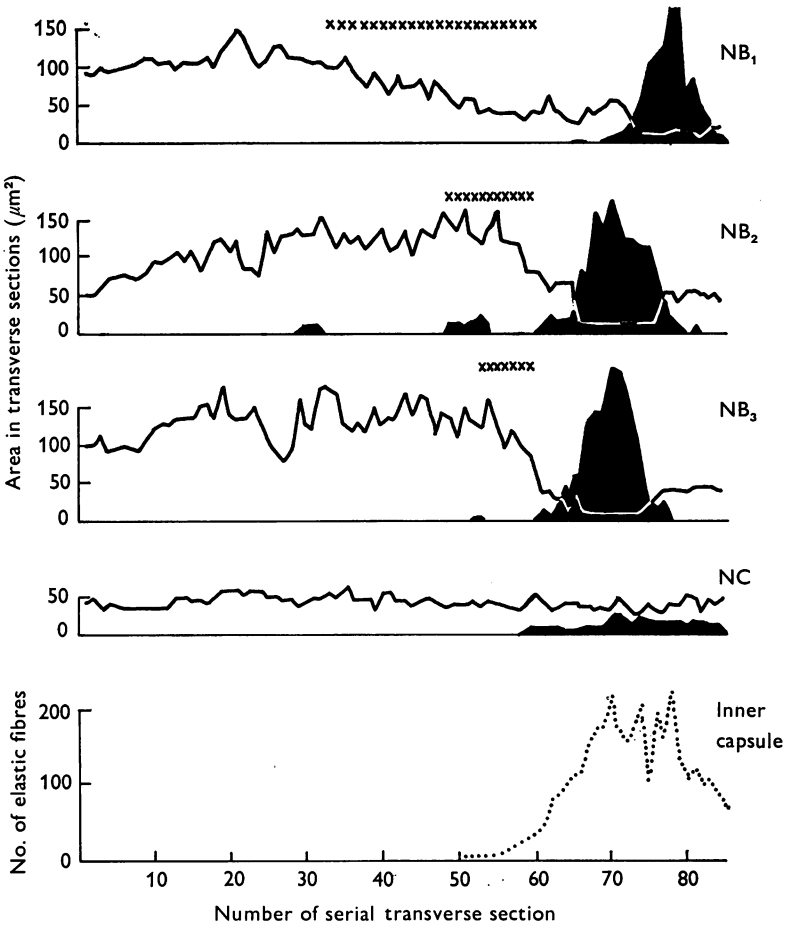

Fig. 2

5. A possible function for branching sensory terminals in frog muscle spindles. By G. BROKENSHA and D. R. WESTBURY (introduced by D. BARKER). *Department of Physiology, The Medical School, University of Birmingham*

Frog muscle spindles respond to stretching with a rapid discharge of impulses, which, if the extension is maintained, becomes slower and more variable as the receptor adapts. Measurements of the interspike intervals have shown that the adaptation following the application of stretch occurs in two distinct phases. At first the impulse train adapts rapidly and is almost regular. Later, the rate of adaptation decreases and the variability increases markedly. In our experiments there was usually an abrupt transition from the first phase to the second. Following stretches of different amplitudes, the transition occurred at about the same mean interspike interval for a

given spindle, but at different times after the beginning of adaptation. The features of the impulse train during adaptation suggest the contribution to the impulse train of several spike generators (Brokensha & Westbury, *J. Physiol.* **232**, 1973).

Small stretches cause the spindles to discharge more slowly than large stretches, and the discharge in each phase of adaptation is less. The discharge in the first phase of adaptation is decreased much more than that in the second. This suggests that, if the phases depend upon separate spike generators, then they may have different amplitude ranges, exhibiting functional differentiation.

The afferent axon of the spindle is derived from several myelinated branches, each connected to multiple unmyelinated terminals situated in the intrafusal muscle fibres (Gray, *Proc. R. Soc.* B, **146**, 1957). Kuroda & Ito (*Proc. Jap. Acad.* **48**, 1972) have shown that each of these branches is capable of initiating action potentials and of discharging the parent axon. Hence it is possible that the separate spike generators lie on different branches of the sensory terminations. Branching sensory terminations usually result in wide receptive fields, but perhaps in the frog muscle spindle the arrangement serves a different function, to allow a large dynamic range.

6. Preliminary physiological studies of chelonian muscle spindles. By M. NAEIJE and A. CROWE (introduced by D. BARKER). *Laboratory of Experimental Physics, Department of Medical and Physiological Physics, Sorbonnelaan* 4, *Utrecht, The Netherlands* (Fig. 3)

Recent morphological studies on chelonian muscle spindles have shown that there are striking structural differences when compared to spindles of other groups. In particular, the equatorial region contains no fluid space and the connective tissue layers of this region wrap closely around the intrafusal muscle fibres and the single sensory neuron. The intrafusal muscle fibres contain no reticular zones or nuclear regions and the myofibrils extend throughout the region of sensory innervation.

Preliminary physiological studies have been made on the response of the chelonian spindle to mechanical stretches in the absence of motor stimulation. The extensor digitorum brevis I muscle of specimens of *Emys orbicularis* and *Pseudomys scripta elegans* was used. Single unit recordings from intact muscle preparations were made by splitting the nerve to the muscle. The time intervals between successive action potentials were recorded in digital form for analysis by a CDC 1700 computer.

Computer print-out records of the instantaneous frequency of firing of a single spindle subjected to ramp stretches at speeds of 0·72 mm s^{-1} and 2·66 mm s^{-1} and height 1·33 mm are shown in Fig. 3. Generally the spindle is observed to fire at a lower frequency than those from other groups except the tonic spindle of the snake. For ramp speeds above about 4 mm s^{-1} no impulses were seen during the release part. For the lower speeds of stretch a steady increase in frequency was observed during the rising phase, but generally fewer impulses were seen during the release phase. During the steady part of the ramp a steady frequency of firing was attained after a period of about 25 s.

7. Types of intrafusal muscle fibre in the Amphibia. By W. K. OVALLE Jr. and R. S. SMITH (introduced by D. BARKER). *Department of Anatomy, University of British Columbia, and Department of Surgery, University of Alberta, Canada.*

Previous histochemical and ultrastructural work has shown that hindlimb skeletal muscles of the Anura are composed of five varieties of extrafusal muscle fibre, three of which are probably types of 'fast' fibre and two are probably types of 'slow' fibre (Smith & Ovalle, *J. Anat.* **116**, 1973). The present study examines the kinds of intrafusal fibre in spindles of these muscles.

While fibre-diameter measurements indicate that they can be divided into two groups – large and small diameter intrafusal fibres – no distinction can be made between them in the histochemical reaction for myosin ATPase. Under alkaline preincubation conditions all the intrafusal fibres stain darkly and homogenously for this enzyme, a reaction similar to that observed in all the extrafusal 'fast' fibres. Ultrastructural examination, however, reveals two varieties of intrafusal fibre, differing mainly in sarcomere and M-band appearance, myofibril organization,

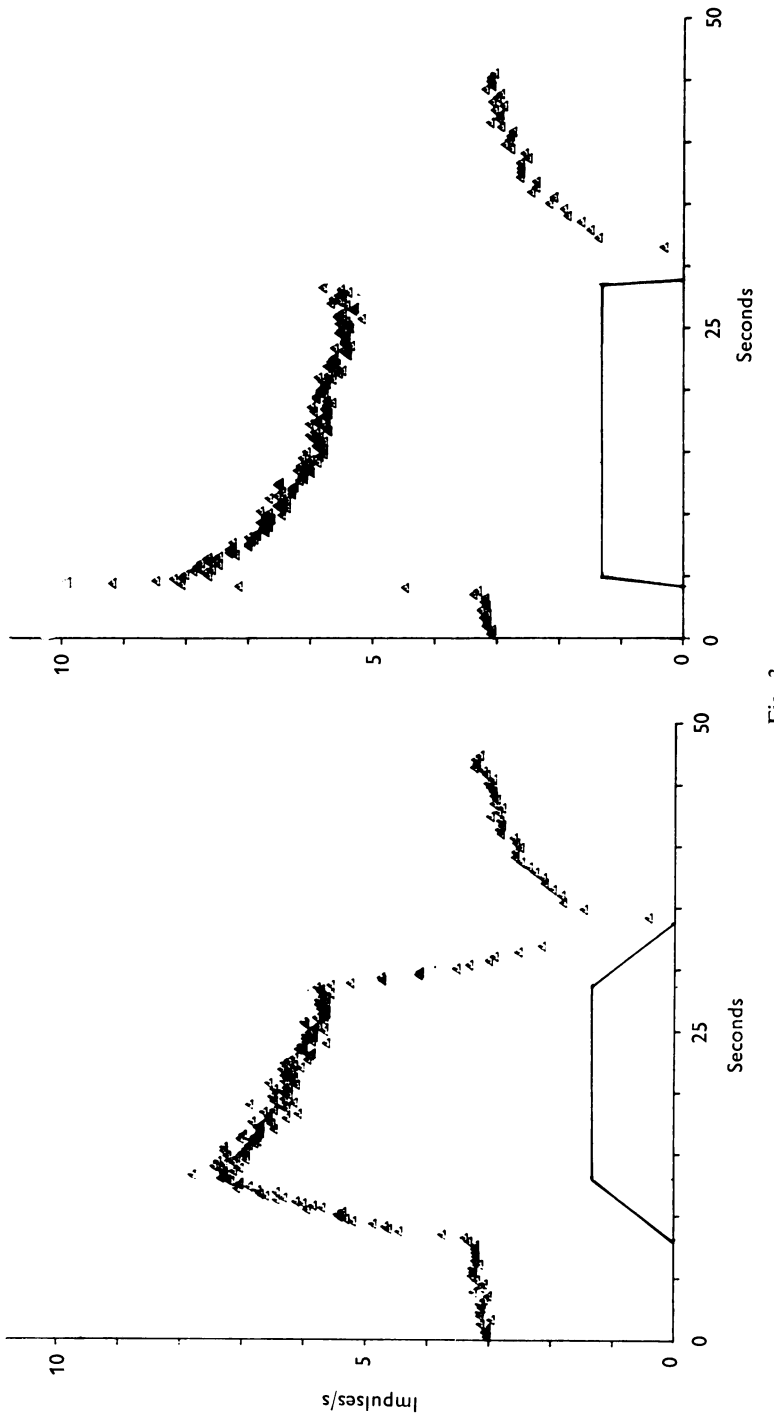

Fig. 3

disposition of the sarcotubular system, and morphology of their motor nerve terminals. The large intrafusal fibre has a structure similar to that of either of two of the fast varieties of extrafusal fibre (types 2 and 3) whereas the small intrafusal fibre exhibits some features characteristic of one of the 'slow' varieties of extrafusal fibre (type 4). Motor nerve terminals on the large intrafusal fibre are of the 'en buisson' type and exhibit postjunctional folds, whereas those on the small intrafusal fibre are smaller, multiple 'en grappe' terminals lacking postjunctional folds. In conjunction with evidence cited in the literature it is concluded that amphibian intrafusal fibres fall into two distinct categories, and that they bear structural features similar to some, but not all, of the extrafusal fibre types. Attempts to match the extrafusal and intrafusal fibres and their innervation into a common organizational scheme, however, should be treated with extreme caution. The unique developmental and functional properties of the intrafusal muscle fibres set them apart quite distinctly from the extrafusal fibres.

Supported by grants from the Medical Research Council and the Muscular Dystrophy Association of Canada.

8. Extracellular material as a modulator of sensory deformation. By U. L. KARLSSON (introduced by D. BARKER). *Departments of Pedodontics and Anatomy, Colleges of Dentistry and Medicine, University of Iowa, Iowa City, Iowa* 52242, *U.S.A.*

The mechanical properties of the frog muscle-spindle intrafusals are generally assumed to determine the deformation of the sensory nerve endings. This concept cannot be unequivocally accepted because several structural problems remain, i.e. the mechanism behind the dual nature (dynamic and static) of the electrical response; the nature of nerve-muscle adhesion; and the strength and resistance of the non-muscular reticular zone when it is subjected to stretch.

Some light may be shed on these questions by inferences based on some previous and recent experimental data. First, extracellular filaments exist primarily around the sensory nerve endings in the reticular zone (Karlsson *et al.*, *J. Ultrastruct. Res.* **14**, 1966). These filaments change from randomized at resting length to periodic organization (extromeres) with stretch (Karlsson *et al. J. Ultrastruct Res.* **36**, 1971). Secondly, stretch appears to induce relatively less sensory deformation in the reticular than in the compact zones (Bendeich *et al.*, in preparation). Thirdly, one of the effects of hypertonic incubation of the isolated muscle spindle is the appearance of numerous muscular protrusions with myofilaments attached to the inside, and extracellular filaments to the outside, of the intrafusal (Karlsson & Ottoson, in preparation). Fourthly, the grooves in the relaxed reticular zone become slits with stretch (Hooker, *et al.*, in preparation).

It is proposed that the muscular protrusions are mechanically linked in series by the extromeres, which must have a mechanical link with the intrafusal. This implies that the intrafusal filaments over the reticular zone are coupled in series by the extromeres which also surround the sensory nerve endings.

The implications of the proposed model are (i) mechanical strength and resistance of the reticular zone may be dependent upon the extromeres rather than the intrafusal; (ii) friction between sensory nerve endings and extromeres forms relative adhesion points necessary for sensory deformation along the nerve chain; and (iii) with stretch, the increased tension in extromeres surrounding nerve endings may inhibit deformation in the reticular zone. Since extromeres are not prevalent in the compact zones, a tentative basis exists for a dual nature of sensory deformation. The proposed model is unique in that it suggests a specific functional role to the extracellular material.

9. Intrafusal muscle-fibre contraction in long-capsule muscle spindles of snake. By C. C. HUNT and R. M. A. P. RIDGE (introduced by D. BARKER). *Department of Physiology and Biophysics, Washington University, St Louis, Missouri, U.S.A.*

Inferior costocutaneous muscles were isolated from *Thamnophis* sp. together with their nerve supply. Using Nomarski optics muscle spindles can be visualized in the intact muscle, usually for most of their length. Intrafusal contraction could be observed in long-capsule spindles following stimulation of single motor axons to either slow-twitch or tonic motor units. Extra-

fusal contraction to stimulation of fast-twitch units precluded observation of intrafusal fibre contraction. Long-capsule spindles also showed contraction on stimulation of the whole muscle nerve when extrafusal contraction was blocked by critical curarization ($< 1 \times 10^{-6}$ g/ml tubocurarine Cl). Intrafusal contraction was photographed at frame rates of 25–100/s. Contractile responses were recorded in the capsular and polar regions. Sensory discharge of the spindle was also recorded from the muscle nerve after interrupting the sensory innervation to other spindles. The contractile responses were similar whether slow-twitch or tonic motor units were stimulated in critically curarized muscles. Sarcomere shortening was clearly maximal at foci where motor terminals could often be seen. In critically curarized muscles contraction occurred on both sides of the region of sensory innervation resulting in longitudinal extension of the sensory terminals. There was often more than one focus toward one pole of the spindle. The extreme poles of the spindle were pulled toward the sensory region. Away from a focus sarcomere shortening and longitudinal translation occurred. Responses to single shocks had a duration of under 200ms at a focus (20° C). Repetitive stimulation showed summation of contractile responses at foci and shortening farther away from foci. Individual motor units showed similar contractile responses, maximal at one or more foci, whether they were slow-twitch or tonic. Under conditions of these experiments contractions were not seen in intrafusal fibres of short-capsule spindles.

10. Response of the short-capsule spindle in reptiles. By Y. FUKAMI and C. C. HUNT (introduced by D. BARKER). *Department of Physiology and Biophysics, Washington University, St Louis, Missouri, U.S.A.*

The sensory response of muscle spindles to stretch usually consists of dynamic and static components, the former being stretch-velocity sensitive and the latter being related to the degree of stretch. It has been assumed that the dynamic sensitivity can be accounted for by the viscoelastic properties of the intrafusal muscle fibre. This hypothesis has recently been challenged by Ottoson & Shepherd (*J. Physiol.* **207**, 1970). They photographed the sensory region of the isolated frog spindle during extension and found no greater displacement during the dynamic phase of stretch.

Because of their simple structure, isolated living reptilian spindles may be visualised in considerable detail using differential interference contrast (Nomarski) microscopy. They, therefore, provide especially suitable preparations for studying the relation between displacement and discharge frequency in response to stretch. Because of its conspicuous dynamic response to applied stretch the short-capsule spindle was mostly used for the present purpose. The other type of spindle, the long-capsule or the tonic spindle, was occasionally examined for comparison.

To identify elements seen in the sensory region of a living spindle Nomarski optics and electron microscopy were conjointly used for one and the same spindle. Some photomicrographs which corresponded to each other were presented. By using a high-speed camera, various elements including outer and inner capsules, sensory endings, and intrafusal muscle elements (nuclei, sarcomeres and granules, etc.) were photographed during ramp-and-hold stretches applied to both ends while recording sensory discharge from the muscle nerve. The speed of the camera was adjusted so that a reasonable number of photographs was taken during the ramp phase of stretch (ramp duration, 0·5–0·13 s; frame rate, 50–18/s). We have observed no consistent phasic component of the displacement of any element in the sensory region which would account for their known dynamic response.

11. Structural studies on two types of snake spindle. By D. J. PALLOT (introduced by D. BARKER). *Department of Physiology, University of Bristol*

Despite the renewed interest in snake muscle spindles comparatively little is known about the structure of their intrafusal fibres, or the details of their innervation. Fukami & Hunt (*J. Neurophysiol.* **30**, 1970) showed differences between the long- and short-capsule spindle intrafusal fibres in the equatorial region, and it has been demonstrated physiologically that the long-capsule spindle intrafusal fibre may receive an innervation from both twitch and tonic extrafusal motor units (Cliff & Ridge, *J. Physiol.* **233**, 1973). This communication reports ultrastructural

and histochemical differences between the two types of intrafusal fibre in their polar regions, and describes the motor and sensory innervation as seen after silver staining and in the electron microscope.

In the polar regions the long-capsule spindle intrafusal fibre sarcomere is characterized by a prominent M line. The fibre contains well delineated myofibrils, and usually a well developed sarcoplasmic reticulum. Occasionally the reticulum is less well developed. In contrast to this there is no well-developed M line in the short-capsule spindle intrafusal fibre, and the sarcoplasmic reticulum is poorly developed. Histochemically the long-capsule spindle intrafusal fibre shows high activities of ATPase, succinic dehydrogenase and phosphorylase. The short-capsule spindle intrafusal fibre shows a low activity of ATPase and phosphorylase and a high activity of succinic dehydrogenase.

Reptilian muscle spindles receive their motor innervation from branches of the axons that innervate extrafusal muscle fibres. The short-capsule spindle intrafusal fibre receives its motor innervation from those axons that innervate tonic muscle fibres with grape endings.

The long-capsule spindle intrafusal fibre receives its motor innervation either exclusively from axons that give rise to grape endings on tonic extrafusal fibres, or from those axons that innervate tonic extrafusal fibres and also from axons innervating twitch extrafusal fibres with plate endings. This confirms the physiological findings.

In both types of spindle the sensory ending is composed of bulbs linked together by small non-myelinated nerve fibres. The links are shorter, and the ending is more highly branched, in the short-capsule spindles.

12. Rabbit intrafusal muscle fibres. By R. W. BANKS and N. T. JAMES. *Department of Human Biology and Anatomy, University of Sheffield*

Early studies on rabbit muscle spindles indicated that they contain only one type of intrafusal muscle fibre according to their morphology (Barker, *Quart. J. micr. Sci.* **89**, 1948; Barker & Hunt, *Nature, Lond.* **203**, 1964) and their myoglobin content (James, *Nature, Lond.* **219**, 1968). Subsequent enzyme histochemical and ultrastructural studies indicated that either two (Spiro & Beilin, *J. Histochem. Cytochem.* **17**, 1969; Corvaja & Pompeiano, *Pflügers Arch. ges. Physiol.* **317**, 1970) or three types (Barker & Stacey, *J. Physiol.* **210**, 1970; Barker *et al.*, *Research in Muscle Development and the Muscle Spindle*, Excerpta Medica, 1972) are present. Confirmatory evidence is presented here which supports the view that there are three types of rabbit intrafusal fibre.

Spindles from several hindlimb muscles were found to contain 4–7 intrafusal fibres. Two types could be identified according to their ultrastructure. (*a*) Fibres which possessed prominent M lines. These contained relatively large mitochondrial volume fractions when analysed using sterological techniques. (*b*) Fibres which did not possess M lines. These contained a significantly smaller mitochondrial volume fraction ($\sim 35\%$ less, $P < 0{\cdot}001$).

In each spindle examined the fibre with the largest equatorial diameter was always a nuclear bag fibre which did not possess M lines. The remaining fibres in each spindle always possessed M lines, but some of these were nuclear-bag fibres and some were nuclear-chain fibres.

The equatorial diameters of the muscle fibres of a spindle, when expressed relative to the diameter of the largest fibre, form a trimodal distribution. Fibres of intermediate equatorial diameter were nuclear-bag fibres and the smallest diameter fibres were nuclear-chain fibres. Relative polar diameters formed a bimodal distribution.

Rabbit intrafusal fibres have previously been classified according to their enzyme histochemical properties as type 1, type 2 or type 3 (Banks, *J. Anat.* **108**, 1971).The relatively short and thin type 1 fibres correspond to the nuclear-chain fibres; type 2 fibres probably correspond to the nuclear-bag fibres which possess M lines. The singly-occurring large type 3 fibre corresponds to the large nuclear-bag fibre devoid of M lines. The type 2 fibres were of greater polar diameter than either of the other types ($P < 0{\cdot}01$ in each case).

13. Change in sarcomere length and sensory spiral spacing in isolated cat muscle spindles during fusimotor stimulation. By I. A. BOYD (Introduced by D. BARKER). *Institute of Physiology, University of Glasgow* (Figs. 4 and 5)

Muscle spindles were isolated from the tenuissimus muscle of the cat and the behaviour of nuclear-bag and nuclear-chain intrafusal fibres was observed during repetitive stimulation of the muscle nerve. Stimulus strength was varied and individual fusimotor axons to the spindle recruited one by one until all of them were active. Measurements were made of changes in sarcomere length and in spacing of the sensory spirals of individual intrafusal fibres. Out of 148 fusimotor axons 89% operated either nuclear-bag or nuclear-chain fibres selectively, while in at least 50%, and probably nearly 80%, of spindle poles the innervation was entirely selective. In 40% of spindles the bag fibres had selective innervation common to all. In 30% of spindles one bag fibre was operated along with the chain fibres. In 20% of spindles the two nuclear-bag fibres were operated independently of each other. Part of one such spindle is shown at rest (Fig. 4A; chain fibres kinked) and during activity (Fig. 4B; 100/s; axons b_1, c_2, b_3, b_4, active; chain fibres straight; vertical arrows show corresponding points). The extension of its primary sensory spirals produced by individual axons is shown in Fig. 5.

Shortening of sarcomeres, maximum 6–25%, occurred at discrete foci in nuclear-bag fibres, and fell to 60% of maximal 100 μm from the focus. The frequency producing maximum contraction varied from 50 to 100/s (mean 76/sec; 37 °C, 33 fibres). The maximal opening of the large primary spiral varied from 4 to 27% and could be quite different for the two nuclear-bag fibres in one spindle (Fig. 5). Contraction in nuclear-chain fibre bundles was more diffuse, maximal at a frequency between 100 and 250/s (mean 153/s, 37 °C, 18 bundles), and produced maximal extension of the small primary spirals of from 8 to 12%.

14. The movement of mitochondria at sensory endings in the muscle spindle. By R. S. SMITH (introduced by D. BARKER). *Department of Surgery, University of Alberta, Edmonton, Canada*

The object of the investigation was to examine the transport of mitochondria in the sensory endings of muscle spindles from *Xenopus laevis*. Isolated, living spindles were examined by dark field microscopy. Histochemical tests for succinic dehydrogenase and glycogen were performed on spindle whole mounts and sections of specimens were examined by electron microscopy.

Dark field microscopy showed that the myelinated sensory axons contained rod-shaped organelles 1–8 μm in length by 0·2–0·3 μm in width, and round organelles 0·2–0·5 μm in diameter. The rod-shaped organelles usually remained stationary, while the round organelles moved with a rapid saltatory motion (about 1 μm/s) both distally, towards the sensory terminals, and proximally. About 10 times as many round organelles were seen travelling proximally as distally. It was estimated that 20 round organelles/min (3×10^4/day) leave the terminal myelinated axon.

The terminal sensory bulbs were identified by their location, size, shape, and their high content of glycogen and mitochondria. Within the bulbs round organelles of size equivalent to those seen in the axon moved in clumps. Individual organelles could sometimes be followed as they moved around the bulbs, and rarely between bulbs, at velocities of up to 2 μm/s. The groups of round organelles were estimated on the basis of histochemical and electron microscopic observations, to be at least 96% mitochondria, the remainder being lysosome-like bodies.

The complete terminal tree was estimated to contain 10^5–10^6 round mitochondria. The observations suggest that this pool of mitochondria may be in dynamic equilibrium with a rapidly moving set of round mitochondria within the axon. If depleted at the estimated rate (3×10^4/day), the terminal pool would be reduced to half in a time of the order of 10 days.

15. Dynamic and static responses of primary and secondary endings of isolated mammalian muscle spindles. By C. C. HUNT and D. OTTOSON (introduced by D. BARKER). *Washington University, St Louis, Missouri, U.S.A.*, and *Veterinärhögskolan, Stockholm, Sweden*

Muscle spindles have been isolated from cat tail muscles and responses to stretch recorded in single afferent fibres from identified primary and secondary endings. Impulse activity was recorded to ramp-and-hold stretch of graded amplitude and velocity. Primary endings exhibited

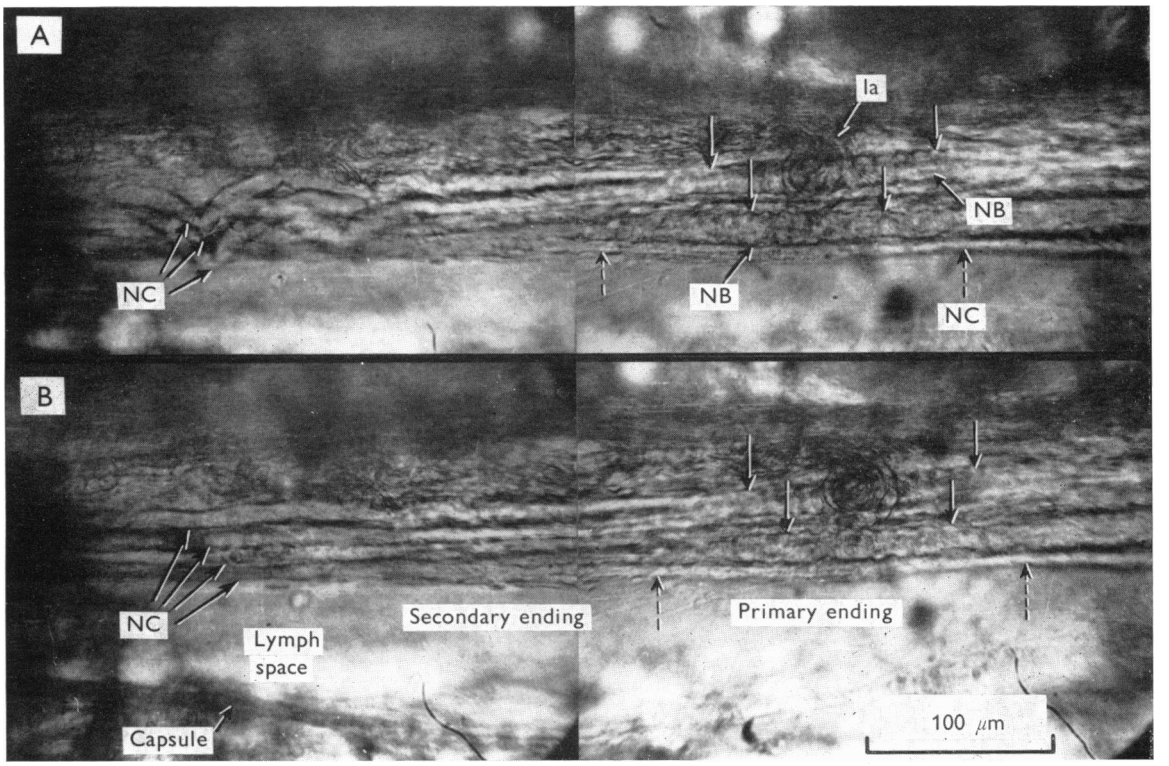
A
Ia
NB
NC
NB
NC
B
NC
Lymph space
Secondary ending
Primary ending
Capsule
100 μm

Fig. 4

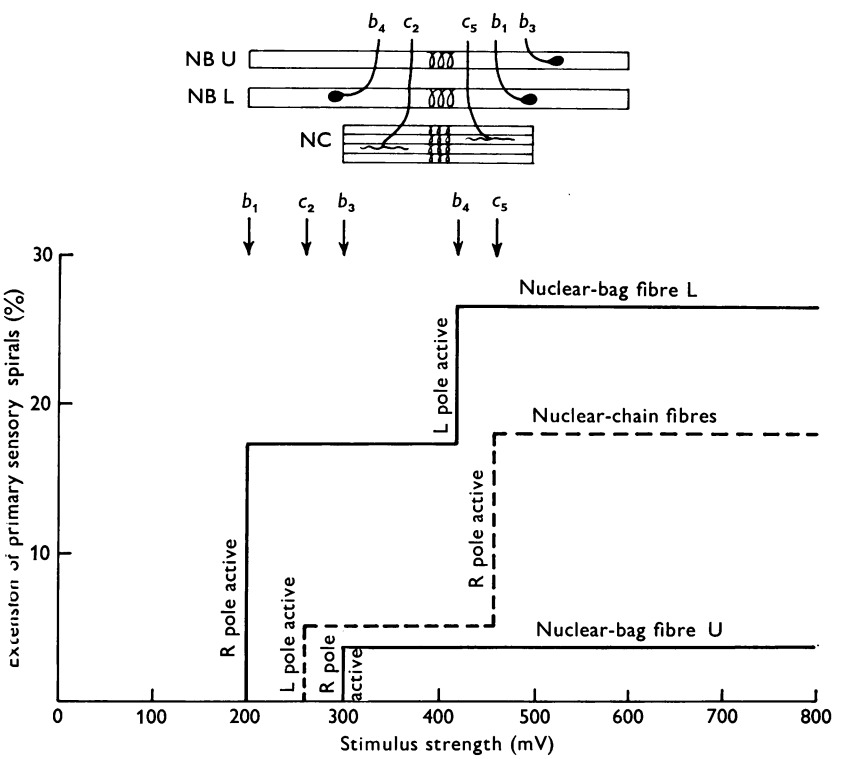
b_4 c_2 c_5 b_1 b_3
NB U
NB L
NC
b_1 c_2 b_3 b_4 c_5
Nuclear-bag fibre L
L pole active
Nuclear-chain fibres
R pole active
R pole active
L pole active
R pole active
Nuclear-bag fibre U
Excursion of primary sensory spirals (%)
30
20
10
0
100
200
300
400
500
600
700
800
Stimulus strength (mV)

Fig. 5

a prominent dynamic response, often with an initial burst, whereas secondary endings showed less dynamic response and no initial burst. Both types of ending gave well maintained static discharge. Following block of impulse activity by tetrodotoxin the receptor potential to identical stretches could be recorded. The receptor potential in primary endings showed a prominent dynamic phase consisting of two components: a rapid depolarization near the onset of stretch, termed the initial dynamic component, and a subsequent slower depolarization called the late dynamic component. The initial dynamic component of the receptor potential is clearly related to the initial burst. They coincide in time and are both diminished by repetitive stretch. The late dynamic component is associated with the subsequent dynamic phase of the impulse discharge. Secondary endings showed a receptor potential with a smaller dynamic component and no initial rapid depolarization except at high velocities of stretch.

While the differences in receptor potential between primary and secondary endings appeared to account for the main features of their impulse frequency responses to ramp-and-hold stretch, comparison of instantaneous frequency plots and receptor potential of individual primary endings suggested that the impulse frequency might depend, in part, on accommodation at the inpulse generating site.

Polarizing currents were applied to the axons of primary endings close to the spindle. Depolarizing currents increased, and hyperpolarizing currents decreased, the frequency of stretch-evoked impulses. Depolarization rectangular current pulses, especially at high current strengths, evoked repetitive discharge showing a relatively small amount of accommodation.

16. The interaction of K^+ and stretching as stimuli for primary muscle-spindle endings in the rat. By G. L. KIDD and C. H. VAILLANT (introduced by D. BARKER). *Department of Physiology University of Liverpool*

Direct measurement, and also estimation based on analysis of venous effluent, show that K^+ accumulates in the interstices of active skeletal muscle. Tetanic stimulation at 50/sec lasting 20 sec raises the extracellular concentration of K^+ in cat gastrocnemius, for example, to 10 m-equiv/l (Hnik *et al.*, *Pflügers Arch. ges Physiol.* **338**, 1973).

The depolarising effect of such a concentration is held responsible for facilitating or initiating activity in cardiovascular and respiratory reflex arcs (Wildenthal *et. al.*, *Am. J. Physiol.* **215**, 1968).

We describe its effect on the primary ending of the muscle spindle in a preparation which allows the bath application or close intra-arterial injection of Krebs' solution with added KCL (Gladden & Kidd, *J. Appl. Physiol.* **507**, 1969).

Increasing the concentration of K^+ from normal for the rat (5 m-equiv/l) to 12 m-equiv/l gives a series of effects which are reversible after washing or a period of normal circulation. These range through facilitation of the effects of stretching, frank excitation summating with stretching, a preclusion of stretch-evoked discharge, to complete inexcitability (Kidd *et al.*, *Physiol. bohemoslav.* **20**, 1971). The facilitation of discharge evoked by stretching the muscle is seen with a K^+concentration of 8 m-equiv/l. The summation of effect of excitation by K^+ and by stretching reaches a maximum without preclusion or interference of activity, at 10 m-equiv/l.

At greater concentrations two effects are seen. The K^+-induced activity is blocked by a further increase in concentration, or the additional stimulus of stretch reduces the activity of the ending. These effects are fully developed at a concentration of 12 m-equiv/l, a concentration that could be expected in muscle approaching terminal fatigue, and would no doubt contribute by its action on the spindle to the associated motor inco-ordination. The powerful afferent drive from the K^+-excited primary ending might also be one cause of cramp, and the failure of the ending to tolerate simultaneous stretching could explain the effectiveness of a brief stretch of a cramped muscle in interrupting the contraction.

17. Evidence for a direct sympathetic reflex modification of muscle-spindle excitability in the rat. By E. G. HALE and G. L. KIDD (introduced by D. BARKER). *Department of Physiology, University of Liverpool*

Stresses such as hypoxia and body cooling cause the release of adrenaline and noradrenaline, which have an influence upon the excitability of neural receptors (Chernetski, *J. Neurophysiol.* **27**,

1964). Considering the muscle spindle, it is still unknown whether this effect is direct upon the ending, or is indirect following an alteration in blood supply. We have used the rat caudal muscle preparation (Gladden & Kidd, *J. Appl. Physiol.* **504**, 1969) to resolve this difficulty. This preparation tolerates ischaemia (Kidd & Vaillant, *Agressologie* **13**, 1972) and can be maintained at a temperature unrelated to the body temperature.

The regularity and frequency of firing of spindle primary endings increased without concomitant change in dynamic index between 20 and 30 s after bilateral carotid artery occlusion or nitrogen breathing. The effect was not blocked by curarization of the muscle ($1:10^4$), and returned to control values within 25 minutes. The enhancement of excitability was unaffected by acute adrenalectomy. Bath application of propanalol (1×10^{-6} M) blocked this enhancement in both the normal and adrenalectomized preparations.

Comparable enhancement was obtained by the bath application of adrenaline (5×10^{-8}M), noradrenaline (5×10^{-7} M), and isoprenaline (5×10^{-6} M). Propranalol (1×10^{-6} M) again blocked these effects, and they could be removed by washing.

The experiments show that naturally released catecholamines have a direct effect on muscle spindles, which can be matched by bathing the muscle with solutions containing these catecholamines at concentrations in the physiological range. The effect is presumably one of β-adrenoceptor excitation and because no change in dynamic index was produced, its most likely site of action is the 'pacemaker' complex of the spindle ending.

As cooling produces catecholamine release in a concentration that increases the discharge frequency from spindles, this could disturb the gain in the servo system involved in muscle movement and could be a factor in initiating the shivering response to cold.

18. Potentiation by stretch of responses from muscle spindles in the cat. By U. PROSKE (introduced by D. BARKER). *Department of Physiology, Monash University, Clayton, Victoria, Australia*

The primary endings to muscle spindles in the soleus muscle of the cat respond to a brief tetanus applied to a single dynamic fusimotor fibre with a characteristic burst of action potentials. The intensity of the burst increases if the muscle is being stretched during the period of stimulation and becomes less if the muscle is shortened. When an alternating sequence of stretch and release at constant velocity is used, potentiation of the response of the spindle to the fusimotor tetanus can be detected for a period of up to 2 seconds following such movements. This potentiation does not depend on the velocity of the movements within the range 1–10 mm/s but requires that the peak-to-peak amplitude of the stretch-release cycle is greater than 0·5 % of the muscle length.

A similar potentiation of the response has not been detected when stimulating a single static fusimotor fibre supplying the same spindle. This makes it unlikely that the potentiation is due to any change in the excitability of the afferent terminals. Rather it suggests a difference in the properties of the intrafusal fibres innervated by static and dynamic axons. Possibly the movement in some way affects the mechanism of activation of the intrafusal fibre. This sort of explanation has recently been proposed for the effect of stretch on the tension developed by active amphibian muscle (Gonzalez-Serratos, Valle & Cillero, *Nature, Lond.* **246**, 1973).

19. γ and β innervation of rat caudal segmental muscles. By B. L. ANDREW and N. J. PART (introduced by D. BARKER). *Department of Physiology, University of Dundee*

These muscles are unusual in two respects. First, although the muscle usually contains two or three muscle spindles, it has only one γ fibre in the muscle nerve; and secondly, the motor units form two separate groups in terms of contraction time. The fast group has a contraction time 10–22 ms, the slow 34–55 ms. In a typical muscle nerve in the mid-tail region; there is one γ fibre two or three efferents to slow motor units, of which one or two are served by β fibres (conduction velocity 20–44 m/s); and five to eight fast units served by α fibres (conduction velocity 34–55 m/s). Our experiments have shown that the γ fibre controls the static sensitivity of the spindles, and the slow-unit β fibres control the dynamic sensitivity of the spindles.

In the lightly anaesthetized animal, the slow units, which are very resistant to fatigue, may be set into tonic activity by manipulation of the tail, and discharge at rates between 5 and

20 impulses/s. These frequencies are effective in changing the dynamic sensitivity of tail spindles, as judged from tests employing ramp-wave stretches and low frequency (3 Hz) sinusoidal stretching movements, The fast units, which have characteristically larger action potentials, appear to be used phasically.

20. Study of β innervation and of β and γ control of isolated muscle spindles in the cat hindlimb
By I. A. BOYD, MARGARET H. GLADDEN, P. N. MCWILLIAM* and J. WARD (introduced by D. BARKER). *Institute of Physiology, University of Glasgow, Glasgow*

Recent electrophysiological work showing that a significant proportion of muscle spindles in certain cat hindlimb muscles receive β innervation (McWilliam, *J. Physiol.* in press, 1974), and studies on static and dynamic fusimotor action in isolated cat muscle spindles with intact nerve and blood supply (Boyd, Gladden, McWilliam & Ward, *J. Physiol.* **230**, 1973), have now been extended. Almost all the motor axons and Ia afferent axons to either the abductor digitis quinta medius (ADQM) or tenuissimus muscles were isolated in 'single unit' spinal root filaments. Beta innervation was sought for using criteria laid down by Bessou, Emonet-Dénand & Laporte (*J. Physiol.* **180**, 1965). The β and γ axons to a spindle were classified as dynamic or static according to their action on the Ia afferent discharge. The spindle was then isolated and the response of its intrafusal fibres to fusimotor stimulation observed directly and recorded on moving film.

Beta innervation was found in 40% of ADQM spindles and 38% of tenuissimus spindles, usually one β axon per spindle, occasionally two; 30% of fast motor axons to ADQM and 14% of fast motor axons to tenuissimus were β axons. Those β axons which were tested all had a dynamic action on Ia afferents. In any one cat the β axon conduction velocities were usually lower than those of the α axons though some overlap between α and β groups did occur. The response of intrafusal fibres to β axon stimulation has been observed. In one spindle the β axon produced contraction at one pole in at least one nuclear-bag fibre, and no nuclear-chain fibre contraction occurred. In another spindle a dynamic β axon produced contraction at one pole of one nuclear-bag fibre only. A summary of the action of fusimotor axons so far studied is tabulated below.

	Type of intrafusal fibre operated		
	Nuclear-bag fibres only	Nuclear-chain fibres only	Nuclear-bag+ nuclear-chain fibres
Dynamic β axon	2	—	—
Dynamic γ axon	4	—	—
Static γ axon	1	5	7

21. Physiological evidence for skeleto-fusimotor axons in various leg muscles of the cat.
By FRANÇOISE EMONET-DÉNAND, LENA JAMI and Y. LAPORTE (introduced by D. BARKER). *Laboratoire de Neurophysiologie, Collège de France, Paris, France*

The observation that repetitive stimulation of some single motor axons elicits both the contraction of extrafusal muscle fibres and an increase in the rate of discharge of spindle primary endings, which persists after selective blocking of extrafusal neuromuscular junctions (Bessou, Emonet-Dénand & Laporte, *J. Physiol.* **180**, 1965), is considered as conclusive physiological evidence for the existence of skeleto-fusimotor, or β, axons. For various technical reasons β axons have so far been demonstrated in this way only in very small muscles.

It has now been possible to find β axons in several large muscles of the leg (flexor hallucis longus, soleus, tibialis anterior, peroneus brevis, peroneus digiti quinti) by limiting the search for them to portions of muscle supplied by small nerve branches. Thirty-eight β axons have been identified (conduction velocity range 44–93 m/s). Some supplied two or even three spindles. Instances of two β axons supplying the same spindle occurred.

* M.R.C. Scholar.

Twenty-nine β axons exerted a dynamic action on primary endings. In several instances a powerful dynamic effect was observed when a γ dynamic axon and a β dynamic axon supplying the same spindle were simultaneously stimulated at low frequencies.

Nine β axons decreased the velocity sensitivity of primary endings. In five cases this static action was attributed to the concomitant contraction of extrafusal muscle fibres, since the action of these β axons became dynamic after selective blocking of extrafusal neuromuscular junctions. Four β axons were classified as genuinely static, because their action remained unaltered when extrafusal contraction was abolished.

22. The motor innervation of normal and reinnervated muscle spindles. By M. C. BROWN and R. G. BUTLER (introduced by D. BARKER). *University Laboratory of Physiology, Oxford*

While the distribution of static and dynamic gamma fibres to intrafusal muscle fibres is not as sharply demarcated as some earlier work had implied, nevertheless recent experiments have shown that the distribution is far from random. Glycogen depletion studies (Brown & Butler, *J. Physiol.* **233**, 1973) indicate that individual γ dynamics always innervate nuclear-bag fibres and individual γ statics always innervate nuclear-chain fibres, but may innervate bags as well. Basically concurring results were obtained by microelectrode studies (Barker *et al.*, *C. r. hebd. Séanc. Acad. Sci.*, **275**, 1972) and by direct observation of living spindles (Boyd *et al.*, *J. Physiol.* **230**, 1973).

The accuracy with which reinnervation occurs also shows that the distribution of the two functional sorts of γ fibre is not haphazard. We have found that regenerated γ fibres are always consistent in their action, following a complete section or crush of the nerve to either the tenuissimus or peroneus longus muscle in the cat. An individual γ fibre has either a static or a dynamic action on all the spindles that it reinnervates. It would seem most likely that each type of γ fibre specifically reinnervates selected sites within the spindle. However, during reinnervation in tenuissimus a high proportion of spindle innervation may be due to β fibres (Brown & Butler, *J. Physiol.* in press, 1974), but we do not find this in peroneus longus.

The question then, is just how typical in the tenuissimus? Most of the recent studies showing non-selective innervation are based on tenuissimus, but it has the lowest γ/spindle ratio (1·3:1) in the cat hindlimb (Boyd & Davey, *The Composition of Peripheral Nerves*, 1968), and we find γ dynamic responses less powerful than those from other muscles. We are therefore attempting glycogen depletion by γ stimulation in peroneus longus, which has a γ/spindle ratio of 7·3:1.

23. Distribution of static and dynamic γ axons to cat intrafusal muscle fibres. By D. BARKER, P. BESSOU, ELZBIETA JANKOWSKA, B. PAGÈS and M. J. STACEY. *Department of Zoology, University of Durham*, and *Laboratoire de Physiologie, Université Paul-Sabatier, Toulouse, France*

Using spindles from cat tenuissimus we have recorded the response of individual intrafusal muscle fibres to γ axon stimulation, and have confirmed the identity of the muscle fibre in one and the same spindle.

The static or dynamic action of single γ axons was identified by observing the effects produced by repetitive stimulation at 100/s on the response of the primary ending to phasic stretch. An intrafusal fibre was impaled with a microelectrode and its response to stimulation by the identified γ axon was recorded. The intrafusal fibre was then marked by electrophoretic injection of a 6% solution of the fluorescent dye Procion Yellow.

After fixation and dehydration the spindle was embedded in Epon. The whole of the impaled pole and the equatorial region were then examined in transverse and longitudinal sections using a combination of light, fluorescence and electron microscopy.

The type of potential (junctional or spike) elicited by dynamic or static γ axon stimulation has been recorded from 18 impaled intrafusal muscle fibres. These were identified in terms of their length, diameter, equatorial nucleation, and myofibrillar ultrastructure.

Static γ axons elicited junctional potentials from 3 chains and 6 bags (3 typical: 1 intermediate: 2 unspecified), and spike potentials from 2 chains and 1 intermediate bag. Dynamic γ axons elicited junctional potentials only from 5 typical bags and one chain. In one experiment the same static γ axon produced a spike potential in a chain fibre and a junctional potential in a bag fibre.

In support of previous evidence (Barker *et al.*, *J. Physiol.* **230**, 1973; Brown & Butler, *J. Physiol.* **233**, 1973) these results show that the terminals of static γ axons are not selectively distributed to chain fibres. It appears that the majority of static γ axons terminate on all three fibre types. The terminals of dynamic γ axons seem to be more selectively distributed.

24. Intrafusal glycogen depletion elicited by β axons in cat spindles. By D. BARKER, FRANÇOISE EMONET-DÉNAND, D. W. HARKER, LENA JAMI and Y. LAPORTE. *Department of Zoology, University of Durham, and Laboratoire de Neurophysiologie, Collège de France, Paris, France*

Using a modification of Edström & Kugelberg's (*J. Neurol. Neurosurg. Psychiat.* **31**, 1968) glycogen depletion technique we have studied the sites of termination of 3 single dynamic β (skeleto-fusimotor) axons that supplied 24 tenuissimus spindle poles, and 1 β axon, possibly static, that supplied 3 peroneus digiti quinti (PDQ) spindle poles.

Single β axons were repetitively stimulated at 40–100/s for periods of 45–90 s with the external iliac artery occluded, and then rested for 30–60 s with re-established blood supply. After the final stimulation period (number ranged from 4 to 8) the muscle was excised and frozen in iso-pentane cooled to -160 °C. Tenuissimus muscles were fixed in absolute alcohol at -40 °C for 3 days and embedded in 'Paramat'. Serial transverse sections were stained for glycogen using the PAS method. PDQ was sectioned frozen and pairs of slides processed to demonstrate glycogen (PAS) and either phosphorylase (P'ase), succinate dehydrogenase (SDH), or actomyosin ATPase after alkali pre-incubation (Alk ATPase).

Normal spindles contain three histochemical fibre types. Typical bags are long and thick and are intermediate in activity for P'ase, SDH, Alk ATPase and PAS. Intermediate bags are slightly shorter and thinner and are low for P'ase, Alk ATPase and PAS, and intermediate for SDH. Chains are high for all four reactions.

The 3 dynamic β axons supplying 12 spindles depleted 16 bags from a total of 26, and 3 chains from a total of 51. A high proportion of intermediate bags (13 out of 14) were depleted as compared with typical bags (3 out of 12). Three typical bags, 12 intermediate bags, and 3 chains were depleted in both poles.

The β axon innervating PDQ depleted the extrafusal type B fibres (small-diameter; low P'ase, Alk ATPase and PAS, high SDH); the intermediate bag and one chain of one spindle pole; and the typical and intermediate bags of a second spindle.

25. Influence of inhomogenous extrafusal mechanics upon the static and dynamic behaviour of primary muscle-spindle endings in the cat: By H.-D. HENATSCH, J. MEYER-LOHMANN, J. SCHMIDT and U. WINDHORST (introduced by D.BARKER). *Physiologisches Institut, Universität Göttingen, 34 Göttingen, W. Germany* (Figs. 6 and 7).

Muscle spindles situated in different parts of a muscle (i.e. cat's extensor digit. long., EDL) respond quite differently to similar muscle stretches. An example of this is given in Fig. 6 of two primary endings, one (A, B) located centrally within the belly, the other (C, D) more peripherally in a near-tendon region of EDL. Curves A and C show the two frequency responses to dynamic ramp stretches; the static frequency-extension characteristics are plotted in curves B and D, respectively.

The present experiments provide evidence that these observed differences are not due to different non-linear sensing properties of the spindles, but are rather caused by mechanical inhomogenities of the surrounding extrafusal structures. Numerous small extrafusal compartments were marked at the muscle surface and their relative length changes were carefully measured both at different steps of static muscle extension, as well as during twitch contractions. In Fig. 7, lower half, the relative length changes under static extension in a central extrafusal region (curve A) and in a peripheral region (curve B) are shown. Similarly the upper half shows the static response characteristics of two spindle primaries that were located in one of either of the

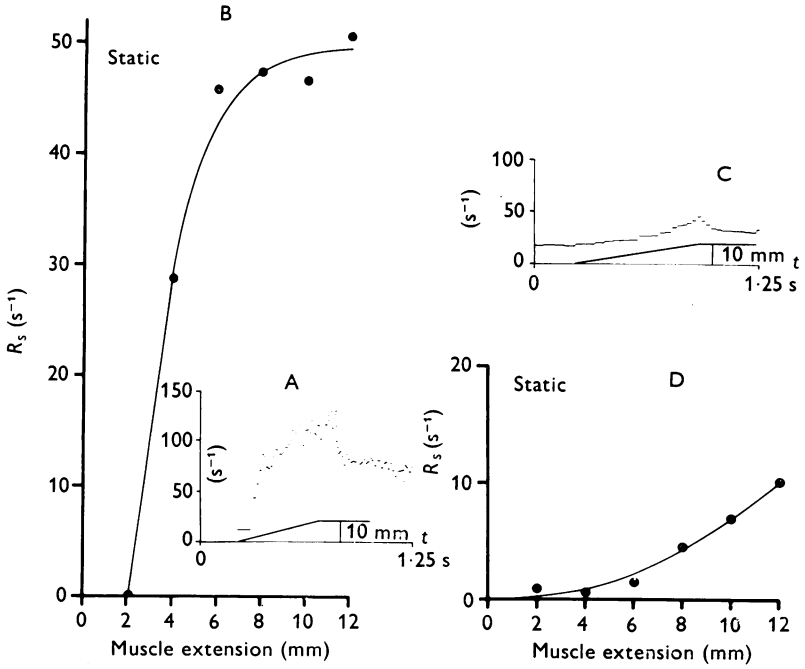
B
Static
50
40
30
20
10
0
R_s (s^{-1})
0 2 4 6 8 10 12
Muscle extension (mm)
C
100
50
0
(s^{-1})
10 mm t
0
1·25 s
A
150
100
50
0
(s^{-1})
10 mm t
0
1·25 s
Static
D
20
10
0
R_s (s^{-1})
0 2 4 6 8 10 12
Muscle extension (mm)

Fig. 6

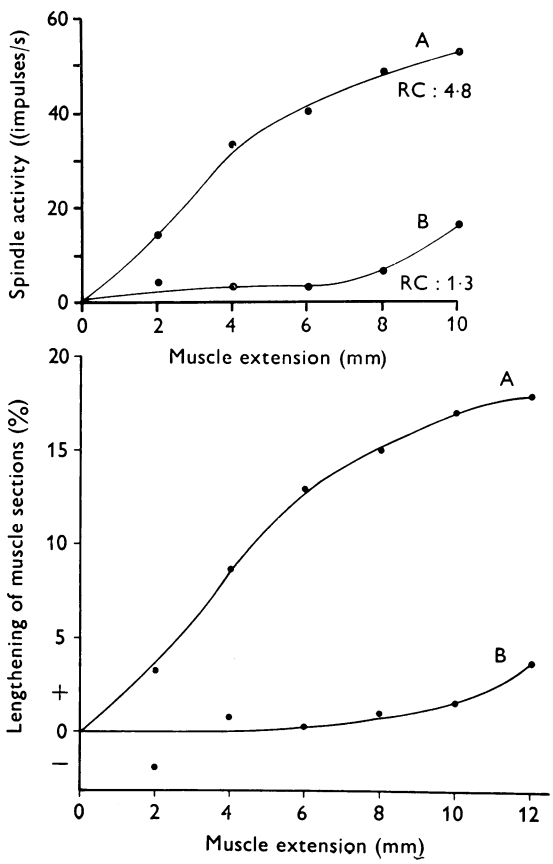
60
40
20
0
Spindle activity ((impulses/s)
A
RC : 4·8
B
RC : 1·3
0 2 4 6 8 10
Muscle extension (mm)
20
15
10
5
+
0
−
Lengthening of muscle sections (%)
A
B
0 2 4 6 8 10 12
Muscle extension (mm)

Fig. 7

two chosen muscular regions. The striking congruence of the two pairs of curves indicates that each spindle measures exactly the different local length changes occurring in the two extrafusal regions.

The complex response of a spindle to a dynamic ramp stretch is composed of several components, as shown by previous workers. These, too, are locally different and can be derived, in principle, from the regional extrafusal events during the ramp stretch. A graphical method is proposed which enables the whole dynamic response of either central or peripheral spindles to be reconstructed from experimental data obtained under purely static conditions.

26. Stretch-evoked inhibition of extensor gamma motoneurons. By C. FROMM, J. HAASE and J. NOTH (introduced by D. BARKER). *Institute of Physiology II, University of Dusseldorf, West Germany*

In the decerebrate cat, stretching of the triceps surae muscle reduced the background activity of 9 out of 25 identified γ-efferents isolated in the medial gastrocnemius nerve (G. M.). This observation confirms the results of Hunt (*J. Physiol.* **115**, 1951). The drop (ranging from 3 to 15 impulses/sec) in discharge frequency became apparent at 8 mm muscle length and increased with the length of the ankle extensors. Cutting the lateral gastrocnemius nerve revealed a marked reduction of this inhibition of G.M. γ-motoneurons, indicating that a considerable amount of stretch-evoked inhibitory input was due to the heteronymous-synergistic muscles. Comparing the responses of the same unit to muscle stretch and to repetitive antidromic stimulation of the parent ventral root of the homonymous muscle nerve, we found that only those γ-motoneurons showing evident stretch-evoked inhibition received recurrent inhibition from the collaterals of the α-fibres.

In the spinal cat, we classified γ-efferents with resting discharges as dynamic, those without resting discharges as static (Alnaes *et al.*, *Acta physiol. scand.* **63**, 1965; Grillner, *Acta physiol. scand.* **77**, 1969). Dynamic γ-motoneurons could not be inhibited by muscle stretch either before or after the injection of 100 mg/kg DOPA. However, 9 static units (out of 16) which responded to DOPA with an initially high discharge rate showed stretch-evoked inhibition. Again, stretch dependent inhibition coincided with antidromic inhibition. But the former was more pronounced than the latter and much stronger than the stretch-evoked inhibition in the decerebrate cat.

We suggest that in the decerebrate cat stretch-evoked inhibition of γ-motoneurons may be mediated by the recurrent collaterals of those α-motoneurons activated by stretch. But in the spinal cat after DOPA a reflex pathway is uncovered, which likely exerts autogenetic inhibition only on the static γ-motoneurons and obviously involves unidentified inhibitory interneurons whose activity seems to be depressed in the decerebrate state.

DEMONSTRATIONS

D. 1 Spindles in some wing muscles of the domestic duck. By M. N. ADAL and SIEW-BOON CHENG CHEW (introduced by D. BARKER). *Department of Zoology, University of Hong Kong*

Muscle spindles have been studied in two dorsal wing muscles of the duck having different action on the pollex, namely, extensor pollicis (EP) and extensor digitorum communis (EDC), with a tendinous branch flexing the pollex. In EP, spindle counts made from longitudinal sections of six muscles show a range of 19-27, mean 23·2, giving a spindle density of 76·7/g. The spindles are randomly distributed in the muscle without any particular reference to the point of muscle-nerve entry. In EDC, similar counts show a range of 37-59 spindles, mean 47·6, giving a spindle density of 119·0/g. The spindles are located primarily in the proximal two thirds of the muscle around the region of nerve entry. In a total of 52 spindles from both muscles studied in transverse section, the fibre-size histogram of intrafusal muscle fibres measured at mid-equatorial region shows a unimodal distribution. However, the fibres are larger and more numerous in EP (number range 5–12, mean 8·7; diameter peak at 13 μm), but with a shorter spindle length (mean 661·8 μm), than in EDC (number range 1–11, mean 4·7; diameter peak at 9 μm; mean spindle

length 1748·1 μm). Nuclear-bag intrafusal muscle fibres, commonly observed in mammalian spindles, have not been encountered. Spindle capsules in EP are thicker and shorter (mean diameter 117·6 μm, length 293·3 μm) than those in EDC (mean diameter 90·2 μm, length 677·0 μm). An elaborate inner capsule system is usually found around individual intrafusal muscle fibres. Only one tandem spindle was observed; it contained two muscle fibres.

D. 2 Innervation of chicken muscle spindles. By N. K. CHIN (introduced by D. BARKER). *Department of Zoology, University of Hong Kong*

Muscle spindles from two fast muscles, posterior latissimus dorsi and lateral gastrocnemius, and two slow muscles, anterior latissimus dorsi and medial gastrocnemius, of the domestic chicken have been studied.

Innervation of the equatorial region of all these spindles is found to be very similar with (i) a single thick afferent nerve providing branches that supply the thickened region of each intrafusal muscle fibre; and (ii) a fusimotor supply of 1–3 fine nerves, which run along the intrafusal muscle fibres to the two ends of the region giving off short delicate twigs that terminate in grape endings.

Fusimotor innervation of the polar regions of spindles from fast and slow muscles differ considerably. In slow muscles, fine nerve fibres, intimately associated with skeletomotor nerves, supply three regions along each pole with grape endings. These are more or less in line with the grape endings on adjacent extrafusal muscle fibres.

On either side of the equatorial region of spindles from fast muscles there is a juxta-equatorial region of grape endings, innervated by fusimotor nerves, which often accompany the sensory supply to the spindle. This region may extend along the entire distal pole, especially in spindles situated near the tendinous end of the muscle. Plate endings are found beyond the juxta-equatorial region of the proximal pole, and are innervated by a nerve fibre that often runs together with skeletomotor nerves supplying plate endings on adjacent extrafusal muscle fibres.

D. 3. Muscle fibre types and collateral innervation in the frog spindle. By R. J. STERLING (introduced by D. BARKER). *Department of Zoology, University of Durham**

Three types of morphologically distinct nerve ending have been demonstrated on frog extrafusal twitch muscle fibres using methylene blue and silver staining techniques. Only one type has been shown to give collateral innervation to intrafusal muscle fibres in extensor longus digitorum IV and iliofibularis muscles. When compared to the findings of Lännergren & Smith (*Acta Physiol. scand.*, **68**, 1966) and Smith & Ovalle (*J. Anat.*, **116**, 1973), it is reasonable to assume that the groups determined in this study correspond to their types 1, 2 and 3 occurring on fast-twitch, intermediate-twitch and slow-twitch muscle fibres (Fig. 8A, C, and B, respectively). Collaterally plate-innervated intrafusal muscle fibres are associated with extrafusal muscle fibres of the slow-twitch type (Fig. 8D).

An examination of the distribution of spindles in transverse sections of iliofibularis muscles revealed that they occur predominantly in the tonus bundle, that is, in proximity to both non-twitch and slow-twitch muscle fibres. Transverse sections of iliofibularis muscle stained for phosphorylase indicate that intrafusal muscle fibres in spindles in the tonus bundle could be divided into two types, one corresponding in profile to extrafusal slow-twitch muscle fibres and the other to extrafusal non-twitch muscle fibres (Fig. 8E, F). Outside the tonus bundle spindles consisted mainly of slow-twitch profile fibres; non-twitch profile fibres were usually absent. This agrees with the work of Brown (*J. Physiol.* **216**, 1971) who showed, using the drug suxamethonium, that about half the spindles in iliofibularis muscle probably lacked grape-innervated muscle fibres. Only one muscle fibre, which occurred in a spindle outside the tonus bundle, showed a profile similar to that of intermediate-twitch extrafusal fibres; none had a profile corresponding to that of fast-twitch fibres. Brown also showed that suxamethonium failed to excite spindles in sartorius muscles. In four sartorius muscles examined, intrafusal muscle fibres of a non-twitch type profile were absent.

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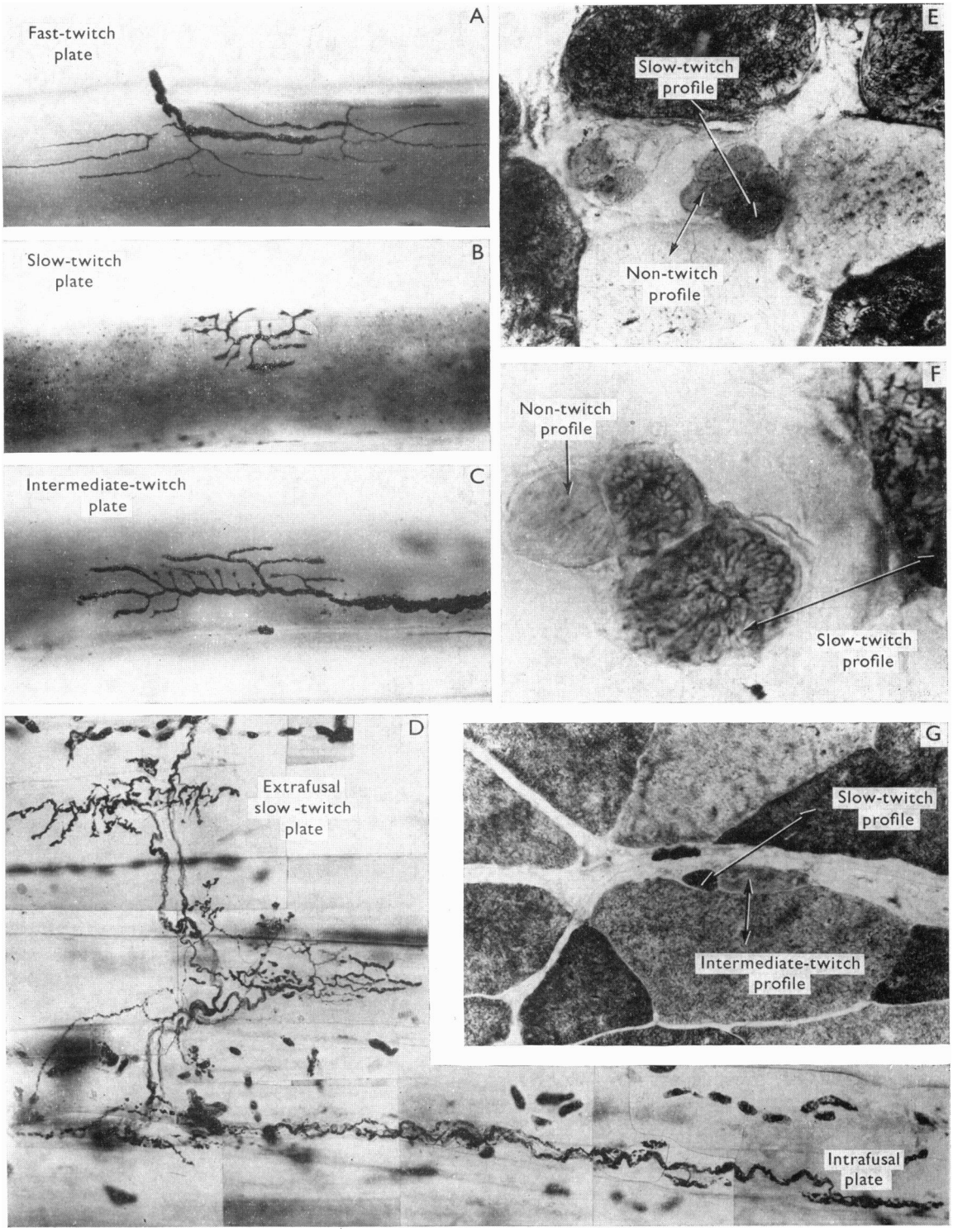
A
Fast-twitch plate
B
Slow-twitch plate
C
Intermediate-twitch plate
D
Extrafusal slow-twitch plate
E
Slow-twitch profile
Non-twitch profile
F
Non-twitch profile
Slow-twitch profile
G
Slow-twitch profile
Intermediate-twitch profile
Intrafusal plate

Fig. 8

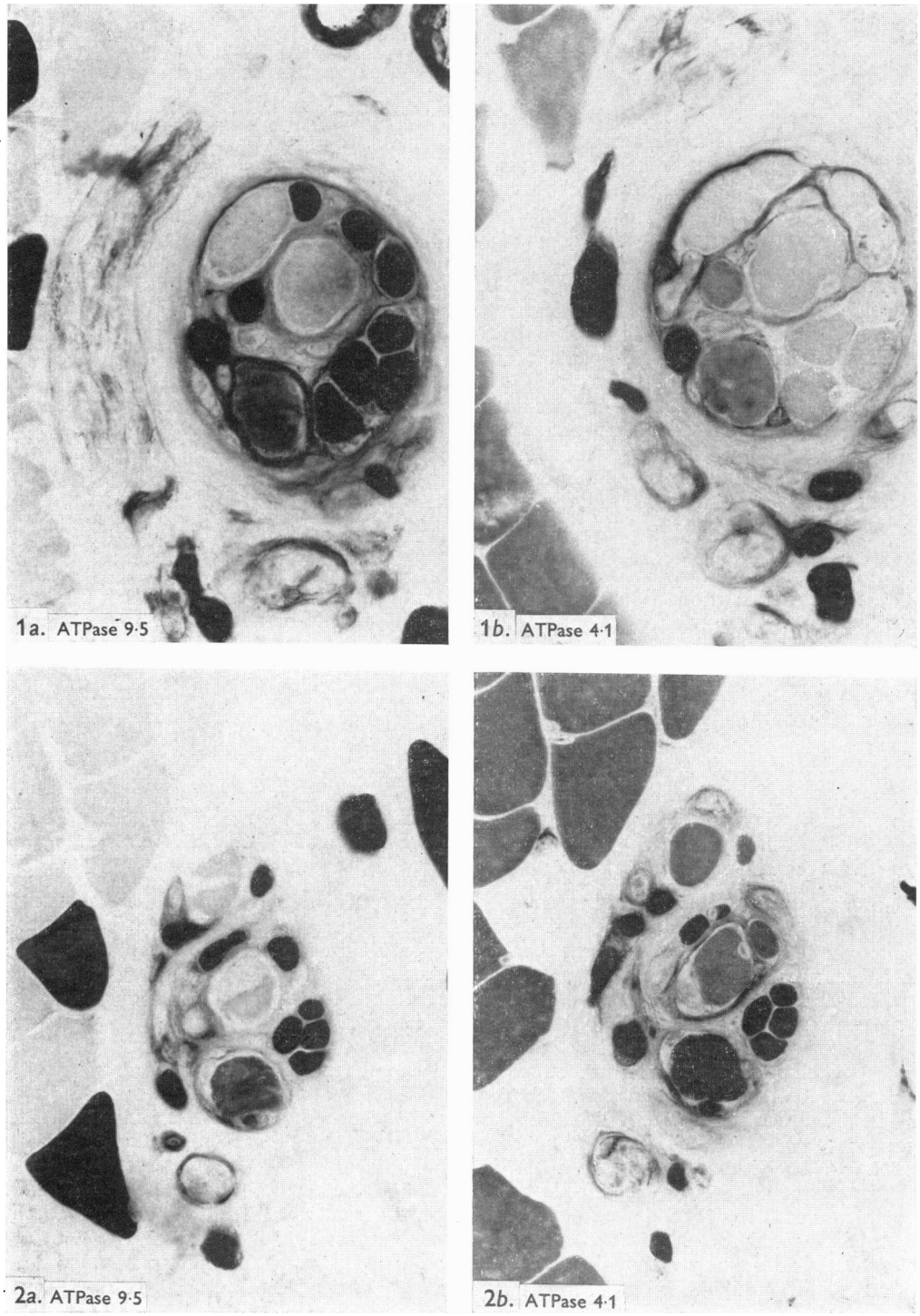

Fig. 9

D. 4. The histochemistry of human intrafusal muscle fibres. By D. G. F. HARRIMAN, P. LESLEY PARKER and B. JEAN ELLIOTT (introduced by D. BARKER). *Neuropathology Unit, University of Leeds* (Fig. 9)

There are differences between the histochemical reactions exhibited by nuclear-bag and nuclear-chain fibres in different species. In the rat James (*Histochem. J.* **3**, 1971) found that nuclear-chain fibres reacted strongly for alkali-stable myosin ATPase, and that nuclear-bag fibres were of two types, those containing both alkali- and acid-stable ATPase, and those containing neither. Ovalle & Smith (*Can. J. Physiol. Pharmacol.* **50**, 1971) found two types of nuclear-bag fibre, those containing acid-stable myosin ATPase only, and those containing acid- and alkali-stable ATPase, in the cat and monkey. Nuclear-chain fibres contained alkali-stable ATPase only.

Little work has been done on human spindles. Spiro & Beilin (*Archs. Neurol. Psychiat. Chicago* **20**, 1969) examined spindles in two infants, and found that nuclear-chain fibres contained alkali-stable ATPase; nuclear-bag fibres were of two types, staining either weakly or very weakly for alkali-stable ATPase. They did not stain for acid-stable ATPase.

We have examined 22 spindles obtained at motor-point muscle biopsy of 16 patients aged 11 months to 59 years. The spindles used appeared to be normal structurally, and the extrafusal muscle was either normal or slightly affected by neuromuscular disease. Cryostat sections cut at 10 μm were serially stained by HE, Gomori, NADH, alkali-stable and acid-stable ATPase, phosphorylase and lipid methods. In our laboratory the most satisfactory reversal of ATPase staining of extrafusal fibres was obtained by pre-incubation at pH 9·5 and pH 4·1. The more commonly used pH 4·3 for the demonstration of acid-stable ATPase produced either partial reversal or no reversal of reaction. The muscles sampled were palmaris longus, flexor carpi ulnaris, vastus medialis of gastrocnemius.

The results indicate that (i) all fibres react strongly for oxidative enzymes (NADH); (ii) there are two types of larger nuclear-bag fibre, one containing little or no acid- and alkali-stable ATPase, and the other containing both varieties fairly uniformly from pole to equator; (iii) nuclear-chain fibres contain alkali-stable ATPase throughout, but are devoid of acid-stable ATPase except at their polar regions, where some react strongly and others moderately (Figs. 9, 1 and 2); (iv) there is no consistent pattern of staining for phosphorylase, both nuclear-bag and nuclear-chain fibres reacting variably; (v) there is a fibre of intermediate size whose staining reactions resemble those of the nuclear-bag fibre.

D. 5. The development of intrafusal fibre types after motor denervation. By JIŘINA ZELENÁ and T. SOUKUP (introduced by D. BARKER). *Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia*

In newborn rats, muscle spindles are only half-formed, containing two nuclear-bag fibres supplied with sensory nerve terminals. Sometimes a third nascent fibre may be present. First fusimotor terminals appear to reach the rat spindles at birth (Barker & Milburn, *J. Physiol.* **222**, 1972; Landon, *J. Neurocytol.* **1**, 1972; Milburn, *J. Cell Sci.* **12**, 1973).

After denervation, the immature spindles of newborn rats degenerate within 10 days (Zelená, *J. Embryol. exp. Morph.* **5**, 1957). When sensory innervation remains preserved and motor innervation of hindlimb muscles is eliminated by ventral root section or by removal of the lumbosacral spinal cord at birth, the differentiation of muscle spindles continues. The mature de-efferented spindles subsequently contain a full complement of intrafusal fibres, usually two nuclear-bag and two nuclear-chain fibres (Zelená, *Progress in Brain Research* **13**, 1964) with ultrastructural characteristics of the equatorial region comparable to those of normal spindles (Zelená & Soukup, *Z. Zellforsch. mikrosk. Anat.* **144**, 1973)

The aim of the present experiments was to establish whether the fibre type characteristics would also develop in contractile regions of intrafusal fibres after permanent motor denervation. The lumbosacral spinal cord was removed in neonatal rats 5–15 hours after birth. Four to eight weeks after the operation different leg muscles of 16 rats with completely paralysed hindlimbs were dissected out and processed for histochemical or electron microscopical investigation (for methods see Zelená & Soukup, *Z. Zellforsch mikrosk. Anat*, **144**, 1973).

Although the de-efferented intrafusal fibres had reduced fibre diameters in comparison with those of normal spindles, they exhibited, as a rule, no gross signs of atrophy or degeneration. In longitudinal sections through the juxta-equatorial and polar zones of de-efferented spindles, ultrastructural differences between intrafusal fibres could be observed similar to those found in normal spindles. One type, presumably nuclear-bag fibres, had confluent myofibrils with a clear H zone and an indistinct M line. The other type, supposedly nuclear-chain or smaller nuclear-bag fibres, had more or less clearly separated myofibrils with distinct M lines and more numerous mitochondria. Triadic or diadic junctions were rare in both types of intrafusal fibre.

In a number of de-efferented spindles, the ultrastructural fibre type characteristics were less conspicuous, but they were nevertheless discernible in 21 out of 25 de-efferented spindles investigated.

In a larger sample of 220 spindles examined in the light microscope on cross sections of de-efferented muscles stained for ATPase activity, the differences in the degree of ATPase activity between intrafusal fibres were marked in the majority of spindles, in contrast to de-efferented extrafusal fibres which were stained rather uniformly.

It can thus be concluded that the fibre type characteristics of intrafusal fibres differentiate in the majority of rat muscle spindles deprived of fusimotor innervation at birth, providing that their sensory innervation remains intact.

D. 6. Quantitative electron microscopical studies on intrafusal myofibrils. By N. T. JAMES and G. A. MEEK. *Department of Human Biology and Anatomy, University of Sheffield*

Two types of intrafusal muscle fibre, nuclear-bag fibres and nuclear-chain fibres, can be differentiated in muscle spindles. Light microscopical techniques have indicated that the equatorial regions of nuclear-bag fibres, unlike those of nuclear-chain fibres, contain faint striations compared with their more strongly stained polar regions. It is generally believed that the weaker equatorial striations reflect a significant reduction in the number of myofilaments in this region. The present morphometric analysis was undertaken to quantify the area occupied by myofilaments in polar and equatorial regions.

Eighteen cat and rat spindles were obtained from lumbrical muscles and serially sectioned for electron microscopy. The total areas occupied by myofilaments (A_A) were estimated in complete intrafusal profiles using point counting techniques (Underwood, *J. Microscopy* **89**, 1968). Significant reductions in the areas occupied by myofilaments were found in the equatorial regions of both fibre types compared with polar regions. These findings are consistent with previous physiological data which suggests that polar regions of intrafusal fibres are relatively viscous whereas their equatorial regions are relatively elastic (Matthews: *Muscle Spindles*, Edward Arnold, 1972).

Estimates of the total areas occupied by myofilaments in whole spindles suggest that their contraction forces, previously measured at $\sim 50\,\mu$N (Diette-Spiff, *J. Physiol.* **193**, 1967), are similar to those of extrafusal fibres (~ 200–300 kN m^{-2}) when expressed as a force per unit cross sectional area.

It is possible that morphometric techniques might provide indirect estimates of tensions in different regions of intrafusal fibres using the values of A_A for equatorial and polar regions. It is suggested that the theoretical methods for analysing muscle fibres with sarcomeres of unequal strength (Hill, *First and Last Experiments in Muscle Mechanisms*, Cambridge University Press, 1970) are also applicable to intrafusal fibres.