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The structure, distribution and innervation of spindles in the extensor digitorum brevis I muscle of the tortoise *Testudo graeca*

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INTRODUCTION

In contrast to mammalian and amphibian muscle spindles, little is known about those of reptiles. According to Regaud & Favre (1904) spindles of lizard and snake are composed of one muscle fibre (monofibrillar) but two types are distinguishable by differences at the equatorial region. One type has a short bulbous capsule which contains a sensory ending restricted to a small region of the muscle fibre; the other type has a long narrow capsule and the sensory ending occupies a larger part of the muscle fibre and branches more extensively.

Szepsenwol (1960) showed that the intrafusal fibres have either plate or grape motor endings, each spindle receiving only one type.

In a review, which included unpublished work of Hunt *et al.* and Proske, Barker (1968) suggested that spindles with long capsules have plate endings and give a tonic response to mechanical stretch, and that those with short capsules have grape endings and their response to mechanical stretch is phasic. Further, Barker suggested that the two types of monofibrillar spindle correspond to the large and small intrafusal muscle fibres in anuran spindles which receive a static/plate and dynamic/grape collateral fusimotor innervation.

Spindles of the alligator are multifibrillar and have from two to five intrafusal fibres (Hines, 1930), with a collateral motor nerve terminating in grape endings on the polar regions. According to Cole (1955) sensory endings are compact and the diameters of intrafusal and extrafusal muscle fibres are approximately the same, the length : thickness ratio being 20 : 1.

Very little is known about chelonian spindles. Giacomini (1898) reported monofibrillar spindles in tortoise muscle but Huber & de Witt (1897) found multifibrillar ones. Apart from preliminary reports (Crowe & Ragab, 1967, 1969) no recent work has been done upon the tortoise spindle, and in view of the conflicting earlier reports and recent knowledge of other reptilian spindles, a more detailed morphological investigation has been undertaken in this animal.

MATERIALS AND METHODS

The spindles were studied in female specimens of *Testudo graeca* weighing 370–710 g. The first extensor brevis muscle was chosen because it and its nerve supply are readily accessible for physiological studies.

Four muscles were used from three of the larger specimens. The muscles were exposed *in situ* until the whole limb had been fixed by immersion in Susa. After removal, they were dehydrated in cellosolve, cleared in toluene, embedded in paraffin, and serial transverse sections were cut at 10 μm. Ten consecutive sections were attached to a slide. Alternate slides were stained with haematoxylin and Curtis's Ponceau S substitute for van Gieson (H & VG), and Masson's haematoxylin-Ponceau–Fuchsin–light green (Masson's triple chrome).

Estimations of length were obtained by counting the number of consecutive sections and correcting for shrinkage. The degree of shrinkage (about 12%) was assessed for each muscle by comparing the lengths of the muscles before fixation and after embedding. It was assumed that all parts of the muscle suffered the same degree of longitudinal shrinkage.

Estimations of diameter of muscle fibres were obtained by taking the mean of values measured in ten consecutive sections. An assessment of transverse shrinkage could not be determined for each individual muscle. Instead, fresh-frozen sections were taken from near the middle of two further muscles and the mean value of the diameters of 500 muscle fibres was found. The remainder of the two muscles was fixed and embedded in paraffin and sections were cut near the mid point. The mean value of 500 fibre diameters was compared with that found in frozen sections. A correction for 25% shrinkage was obtained and applied to all muscle fibre diameters measured.

A detailed study of 27 spindles was made from the serial sections.

Two more muscles, together with two specimens of rat soleus muscle and two specimens of frog gastrocnemius muscle, were also embedded in paraffin. The muscles were not subjected to detailed study but sections were stained by either the orcein method, Verhoeff's method or Hart's method for the detection of elastic tissue.

Teased preparations were obtained from muscles stained by the Barker & Ip (1963) modification of de Castro's silver method, or by a methylene blue method similar to that described by Boyd (1958) in which, instead of perfusing the muscles with methylene blue in Krebs's solution at a pH of 6·6, extra KH_2PO_4 was added to reduce its pH to 5·6. Although perfusion was standardized the quality of staining was unpredictable and variable. Sometimes no endings were stained, sometimes only the sensory endings were stained and at other times all endings were stained.

Since differential denervation was not attempted, identification of sensory and motor nerves to the spindle was made according to the following criteria:

(*a*) Sensory endings were assumed to have some resemblance to sensory endings in spindles of other species; motor endings were assumed to resemble nerve endings of extrafusal chelonian muscle fibres.

(*b*) Sensory nerves would not be branches of nerves to the extrafusal fibres, but motor nerves might be.

(*c*) In those preparations in which extrafusal motor endings were not stained, only one nerve was seen to enter the spindle whose endings were visible. This was regarded as a sensory nerve on the assumption that staining of intrafusal motor endings was unlikely.

(*d*) In silver preparations where the capsule was visible, nerve endings within the capsule were considered to be sensory, and those at the polar regions motor.

(*e*) Studies of serial sections showed that the diameter of each intrafusal muscle fibre had two peak values which roughly corresponded to the limits of the capsule. In some preparations the capsule could not be clearly seen, but if a nerve ending was found to lie between the two positions of peak diameter, it was considered to be sensory.

RESULTS

Structure of the muscles

The EDB I muscle of the tortoise is shaped like a truncated cone split down the axis; thus transverse sections of the muscle are D-shaped. The dorsal surface is convex and the under surface is slightly concave where it overlies bone. The thicker proximal end is attached to the fibula and the thinner distal end to the claw.

The lengths of the four muscles, calculated from the number of consecutive slides in which myofibrils appeared, are given in Table 1. Lengths of individual extrafusal muscles fibres were not measured but the distribution of their diameters is shown in Fig. 19. About 75 fibres were measured in a section taken from near the mid point of each muscle and a histogram was made of the 300 measurements. The mean value was 27·3 μm; S.D. 5·71 μm.

Distribution and position of the spindles in the muscles

The numbers of spindles found in individual muscles that were serially sectioned are shown in Table 1. There is a wide variation in the number of spindles in muscles of similar size, and muscles C and D from the same animal contained 10 and 4 spindles respectively.

No estimate of numbers of spindles could be made for teased preparations: some may have been lost during the teasing and many preparations were taken from smaller animals.

The spindles did not occupy consistent positions in the muscles, but epimysial insertions of their proximal ends were on the convex surface and their distal ends were on the concave surface, and they were frequently found near blood vessels, as in Fig. 1. In passing through the muscle, spindles usually crossed its long axis, i.e. if the proximal end were to the left, the distal one would be to the right of the axis, or vice versa. Most of the capsules were rather deep in the muscle and were usually contiguous with a trabeculum of perimysium.

General structure of the spindles

Table 1 gives the details of the spindles found in the four serially sectioned muscles. The mean spindle length was 9·3 mm within a range of 6·9–11·4 mm. The mean number of intrafusal fibres was 10·4 within the range of 2–17.

The mean position of the mid point of the capsule from the proximal end of the spindle was 4·5 mm. As indicated in Table 1, many spindles had capsules which were much nearer to one end of the spindle than the other. For instance, the capsule of spindle A4 was 1·9 mm from the proximal end, but spindle B2 has a capsule that was 7·5 mm from the proximal end.

Two double tandem spindles were observed. Spindle C4 consisted of 17 intrafusal fibres of which 12 passed through both capsules, three passed through the distal

capsule only and the other two passed through the proximal capsule only. Spindle D6 contained eight fibres; all passed through the proximal capsule and three fibres plus the two arms of the branched fibre passed through the distal capsule.

Table 1. *Principal data of 27 muscles spindles studied in the serial sections of four muscles*

(In the case of the tandem spindles, data for both capsules are included.)

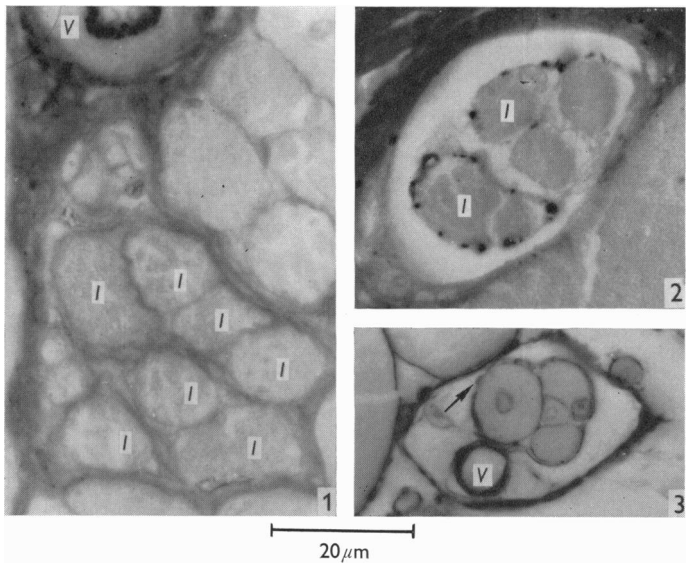
	Spindle number	No. of intrafusal muscle	Spindle length (mm)	Length of capsule (mm)	Distance of mid-capsule from proximal end of spindle (mm)
Muscle A. Length	A1	7	9·5	1·1	4·7
14·1 mm. From	A2	13	10·3	1·3	5·5
left leg of animal	A3	12	10·2	1·0	2·8
No. I	A4	10	10·2	0·9	1·9
Range		7–13	9·5–10·3	0·9–1·3	1·9–4·7
Mean values		10·5	10·05	1·1	3·7
Muscle B. Length	B1	7	11·4	1·8	4·9
15·4 mm. From	B2	11	9·4	1·0	7·5
left leg of animal	B3	3	9·4	0·7	7·2
No. II	B4	8	9·3	1·3	4·0
	B5	8	10·5	0·5	3·5
	B6	14	11·0	0·8	2·5
	B7	16	9·9	1·3	5·8
	B8	7	10·2	1·4	6·3
	B9	4	10·1	1·1	6·2
Range		3–16	9·3–11·4	0·5–1·8	2·5–7·5
Mean values		8·7	10·13	1·1	5·3
Muscle C. Length	C1	13	9·6	1·7	4·7
12·9 mm From	C2	13	10·2	1·9	4·9
left leg of animal	C3	11	9·6	0·8	3·8
No. III	C4 (tandem)	17	8·3	1·0	1·1, 5·9
Range		11–17	8·3–10·2	0·7–1·9	1·1–5·9
Mean values		13·0	9·43	1·22	4·8
Muscle D. Length	D1	9	8·2	0·9	6·5
12·5 mm. From	D2	2	8·7	0·6	6·2
right leg of animal	D3	17	6·9	0·8	5·6
No. III	D4	10	8·5	0·8	5·1
	D5	17	8·6	1·3	2·0
	D6 (tandem)	8	7·9	0·8, 0·6	2·2, 3·5
	D7	16	9·7	1·1	2·2
	D8	14	8·0	0·9	4·9
	D9	11	9·5	1·1	5·0
	D10	6	7·0	1·3	2·9
Range		2–17	6·9–9·7	0·6–1·3	2·0–6·5
Mean values		11·0	8·29	1·02	4·6

In several cases, especially where a spindle contained a large number of muscle fibres, they tended to spread out as they approached the poles. Although the spreading might be confined to one end, sometimes the spreading was minimal; often fibres

tended to form a line in transverse section (Fig. 12); at other times they separated into two or more groups.

No elastic tissue was found in spindles of the tortoise although it was present on the internal surface of the blood vessels (Fig. 1) and in spindles and blood vessels of rat and frog muscle (Figs. 2, 3).

At least one blood vessel was seen in each spindle; usually there were two or three in the outer layers of the capsule and between intrafusal fibres in this region (Fig. 13).



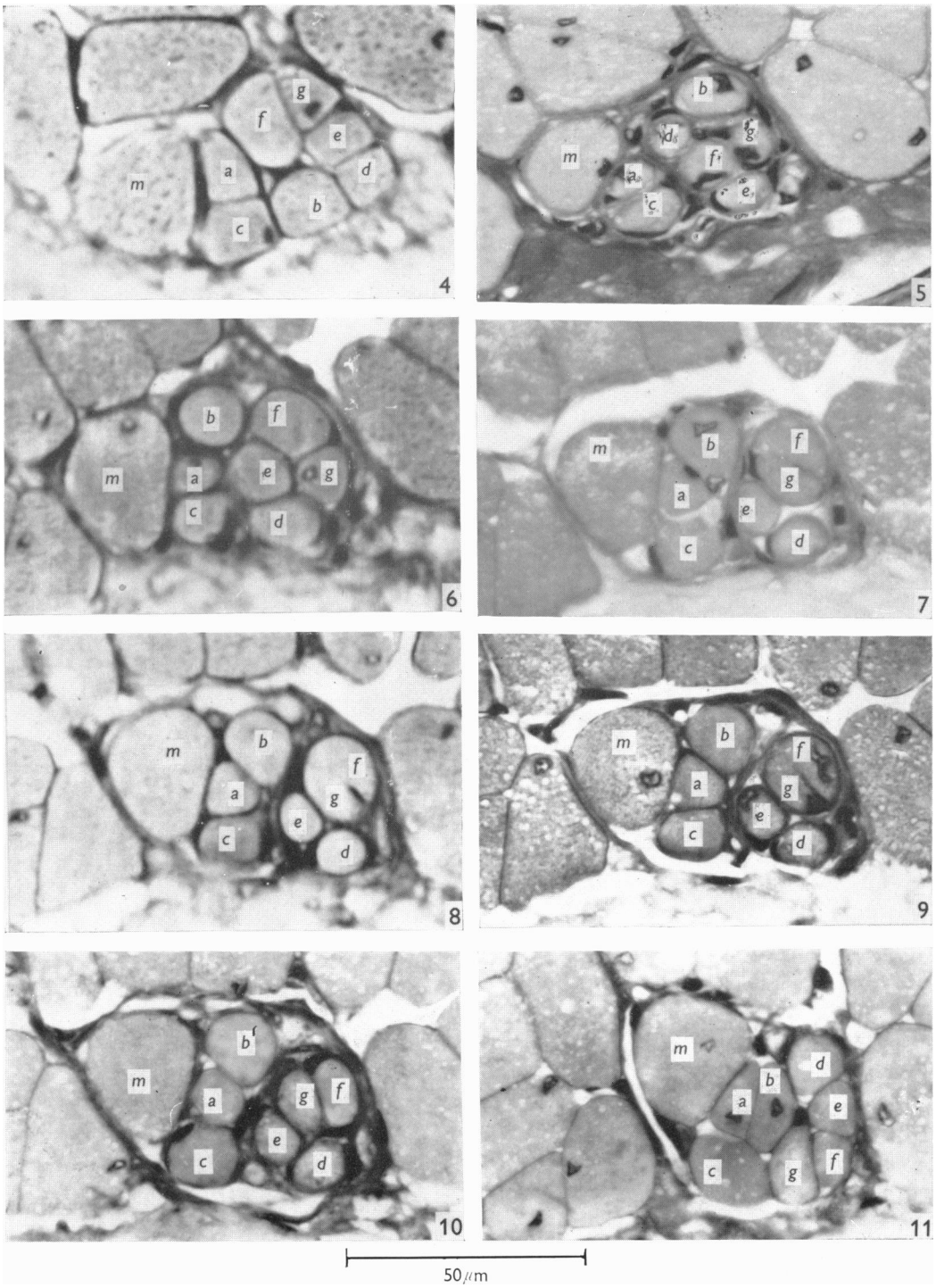
Figs. 1–3. Transverse sections through the equatorial regions of tortoise, rat and frog spindles, respectively, which have been stained to show the presence of elastic tissue. It appears as dots on the periphery of the intrafusal fibres (*I*) of the rat spindle, and appears as finer dots, two of which are indicated by arrows, on the intrafusal fibres of the frog spindle. None appears on the intrafusal fibres (*I*) of the tortoise spindle. It is prominent in the lumen of the blood vessel (*V*) of the tortoise section and can be seen in the blood vessel (*V*) of the frog spindle. Orcein stain.

Structure of the capsule

Each capsule seen in serial sections consisted of layers of connective tissue and each intrafusal fibre also had a sheath consisting of one or two layers. Several fibres were sometimes grouped together in a two or three layered common sheath, and finally the complete capsule might have an outer sheath consisting of two to four layers of connective tissue. Each intrafusal fibre was thus separated from the outside of the spindle by five to eight layers of connective tissue. The spindle shown in Fig. 14 consisted of two groups of four and five intrafusal fibres, but grouping tended to change at different points along the capsule. For example, in the spindle shown in Fig. 5, the intrafusal fibres *a* and *c* formed one group, but in other regions of the same capsule (Figs. 9–10) *a*, *b* and *c* formed another.

The outer sheath of connective tissue often embraced a single, thicker extrafusal fibre (Figs. 4–12) and sometimes it enclosed several (Fig. 13).

At the polar regions connective tissue around intrafusal muscle fibres was no


4
5
6
7
8
9
10
11
50 μm

thicker than the endomysium of extrafusal fibres (Figs. 4, 12). In these regions it was often impossible to distinguish intrafusal from extrafusal muscle fibres unless the former could be traced through into the capsular region in serial sections.

The extent of the capsule could be measured quite easily, although, in some cases, intrafusal muscle fibres did not enter or leave the capsule simultaneously. The lengths of the capsules given in Table 1, corrected for shrinkage, ranged from 0·5 to 1·9 mm, with a mean of 1·05 mm.

The connective tissue of the capsule was closely approximated to the intrafusal muscle fibres (Figs. 1, 4–14), in sectioned and teased preparations, and the bulbous fluid-filled space seen in rat (Figs. 2, 15) and frog spindles (Figs. 3, 16) was never found in the tortoise.

Structure of the intrafusal muscle fibres

On the basis of length, diameter, nuclear density and myofibrillar density, no evidence was found to suggest that there was more than one type of intrafusal muscle fibre.

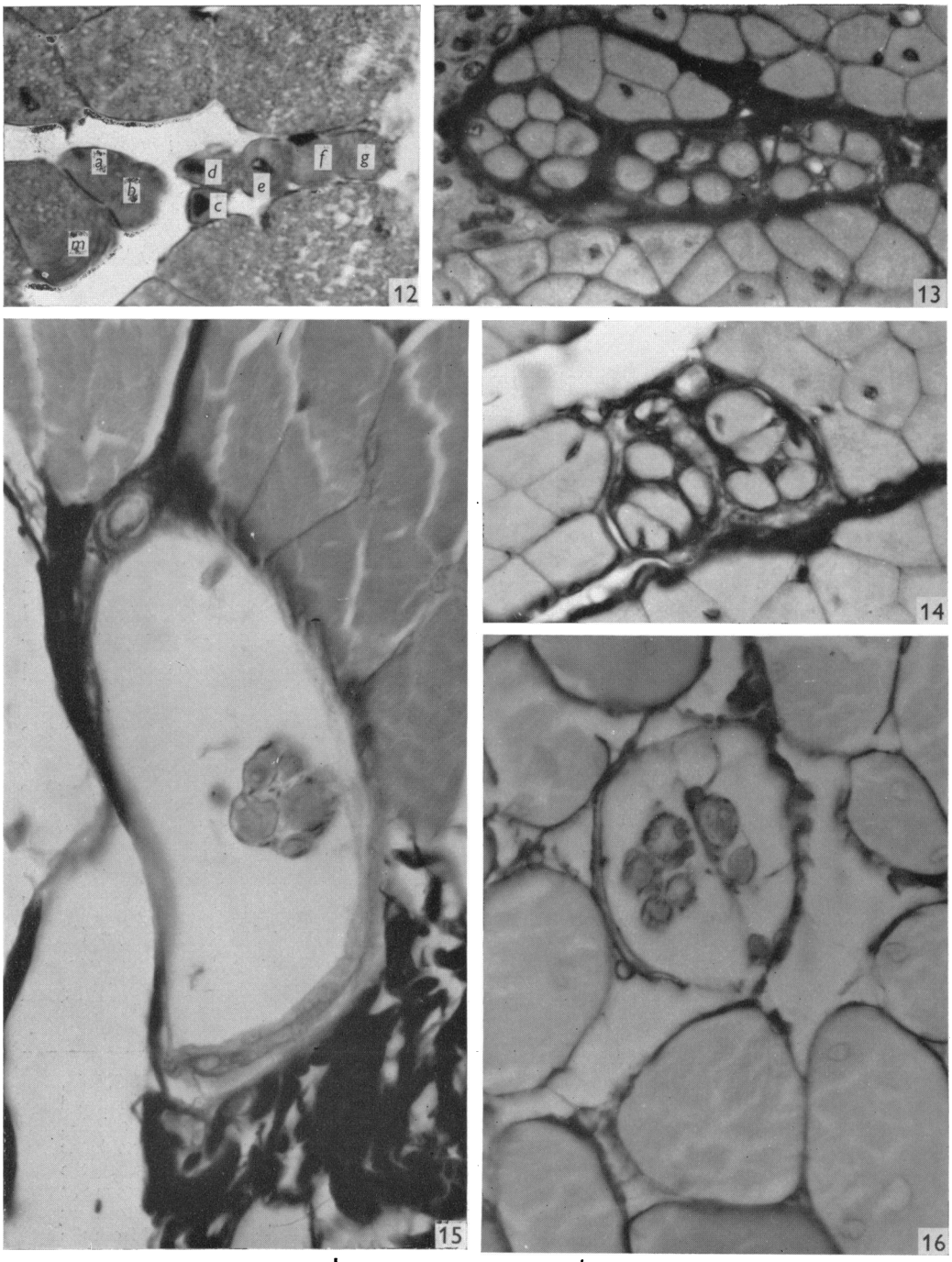
The length distribution of the 282 intrafusal fibres was unimodal (Fig. 17). The mean length was 9·1 mm; S.D. +1·39 mm. Ninety-three per cent of all the intrafusal muscle fibres extended over more than 95% of the total length of the spindles.

The fibres were not of uniform diameter over their length. Plotting fibre diameter against distance along the fibre gave a double-peaked curve of which Fig. 18 is a typical example. Fibres enlarged in diameter as they approached the capsule and attained a maximum just prior to the point of entry. Within the capsule, diameter decreased to a minimum about halfway through. It then increased to reach the second maximum just beyond the point of emergence.

Two points were chosen on each polar region of each fibre midway between the end of the fibre and the nearest part of the capsule. The diameter of the fibre was measured on ten consecutive sections at these two points and the mean value calculated. The values for each of the intrafusal fibres were plotted in a histogram (Fig. 19). The mean of the 282 values was 10·6 μm; S.D. 2·30 μm.

The cross-sectional shape of intrafusal muscle fibres varied at different points along the spindle. In the capsular region (Figs. 5–10, 13, 14) it was circular, ellipsoidal or kidney-shaped, and spaces between fibres were filled with connective tissue. At the polar regions (Figs. 4, 11, 12) fibres tended to be polygonal in shape because there was less connective tissue around them and adjacent fibres pressed against each other.

Figs. 4–11. Serial transverse sections of a single tortoise spindle at distances of −0·5, 0, 0·3, 0·43, 0·44, 0·45, 0·47 and 0·9 mm respectively from the mid-capsular region. Intrafusal fibres are lettered (*a*)–(*g*). An extrafusal fibre (*m*) is included in the outer connective tissue sheath of the spindle. Note the apparent fusing of the fibres (*f*) and (*g*) in Figs. 7–9. The section of Fig. 4 is outside the capsule and the connective tissue around intrafusal fibres is no thicker than that surrounding the other fibres. The section of Fig. 5 is at the mid-capsular region when the intrafusal fibres are at their thinnest, and the connective tissue is arranged so that fibres (*a*) and (*c*) are grouped together in contrast to the sections of Figs. 7–10, where the fibres (*a*), (*b*) and (*c*) are separated from the remainder. The section of Fig. 11 is near the edge of the capsule and very little connective tissue is present around the intrafusal fibres. All the sections are stained with Masson's triple chrome except Fig. 5, which is H & VG.


a
b
c
d
e
f
g
12
13
14
15
16
50 μm

It was possible to plot the distribution of nuclei along each intrafusal fibre. The combined distribution for the eleven fibres of spindle D9 (Fig. 10) was typical of all the fibres studied. They were fairly evenly distributed along the extracapsular length of the fibre; density was slightly higher in the intracapsular portion. No myotube region was observed in any fibre, and there was no apparent structural difference between polar and equatorial nuclei and between nuclei of intrafusal and extrafusal fibres.

With Masson's stain, each fibre appeared to be stained throughout its entire length; myofibrils were continuous through the capsular region and were not replaced at any point by elastic or connective tissue. They took up the stain to the same extent as the extrafusal fibres.

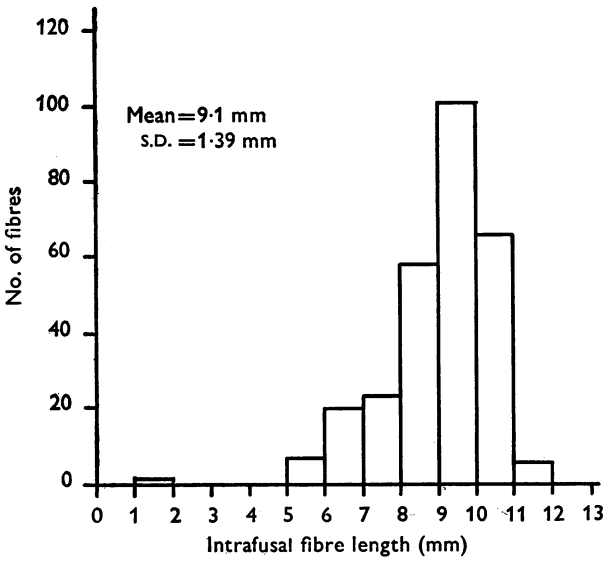


Fig. 17. A histogram of the total lengths of 282 intrafusal muscle fibres observed in serial sections.

Two branching intrafusal fibres were found. In spindle D4 the fibre was single for most of its length, including the intracapsular part, but it branched on emergence from the capsule. The two arms of the branched part were of equal length, 2·9 mm. The fibre in Spindle D6 was single through one of the capsules; it branched at the point of emergence, and both arms went through the second capsule. The two arms were of equal length, 4·6 mm.

Fig. 12. Same spindle as Figs. 4–11 with identical labelling of intrafusal fibres, but section taken 3·7 mm from the mid-capsule to show the spreading of the intrafusal fibres at the poles. Masson's triple chrome.

Figs. 13, 14. Transverse sections of tortoise spindles at the capsular regions to show the separation of the intrafusal fibres into distinct groups, and, in the case of Fig. 13, to show the inclusion of a bunch of extrafusal fibres in the outer connective tissue of the spindle. Masson's triple chrome.

Figs. 15, 16. Transverse sections of rat and frog spindles respectively to demonstrate the size of the capsule with respect to the intrafusal fibres and to show (in contrast to the tortoise spindles) the presence of a large fluid space within the capsule. Orcein.

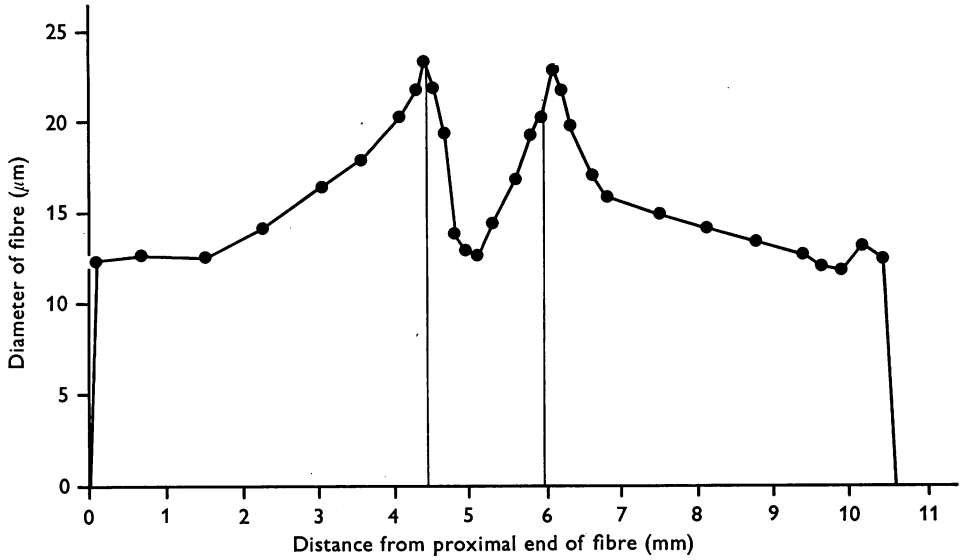


Fig. 18. A graph to show the variation in intrafusal fibre diameter at different points within the spindle. The measurements were taken on a single fibre and each point represents the mean of values measured on consecutive 10 μm sections. The shape of the graph is typical for intrafusal fibres and the two peak values occur just outside the limits of the capsule as indicated by the two vertical lines.

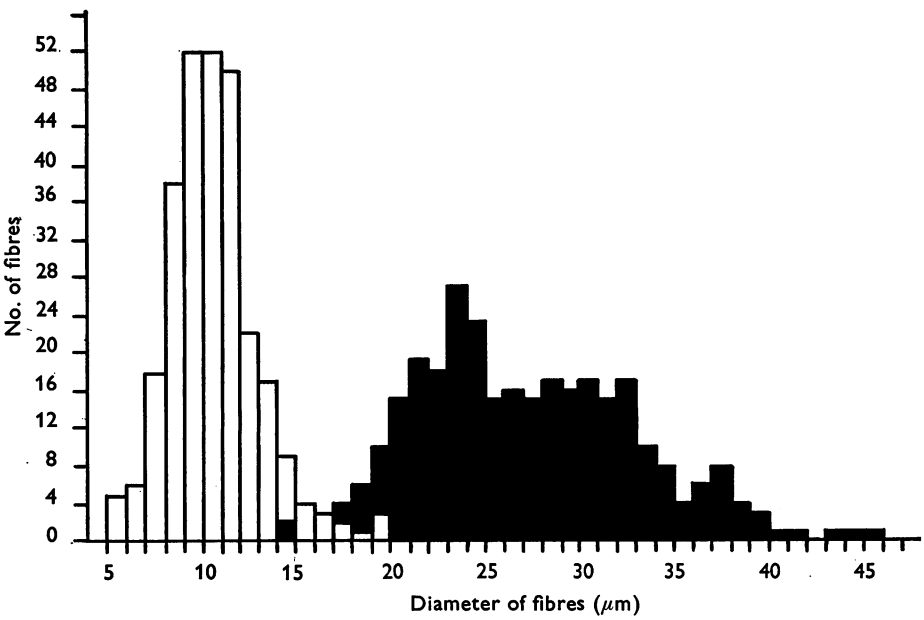


Fig. 19. A histogram of the diameters of 282 intrafusal (unshaded columns) and 300 extrafusal muscle fibres (shaded columns). The intrafusal fibre diameters were measured at points halfway between the end of the fibre and the edge of the capsule. The extrafusal fibres were measured in sections taken from the mid-regions of the four muscles that were sectioned serially. □, 282 intrafusal fibres, mean diameter 10·6 μm, S.D 2·30 μm. ■, 300 extrafusal fibres, mean diameter 27·3 μm, S.D. 5·71 μm.

Since only light microscopy was used in the present studies it is impossible to be certain whether true bifurcation of the fibres occurred. For the purposes of constructing histograms and summaries, an intrafusal fibre and its branches were treated as one unit.

Several instances were observed where intrafusal fibres within the capsule seemed to fuse together for 20–40 μm. Fusion of fibres was observed in most spindles; an example is shown in Figs. 6–10. Sometimes it was seen in more than one pair of

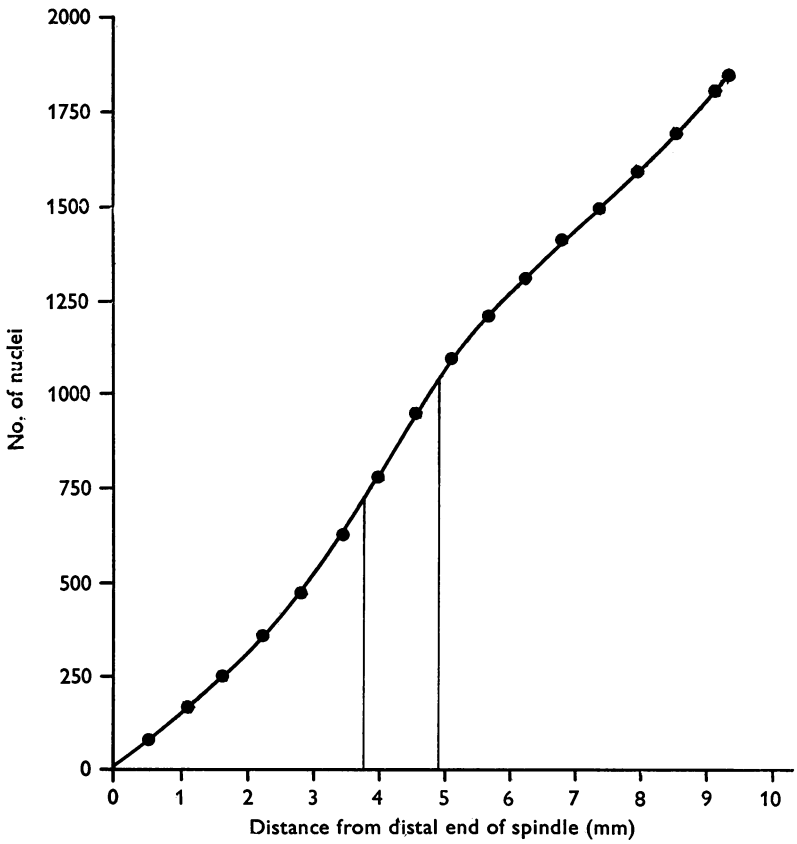


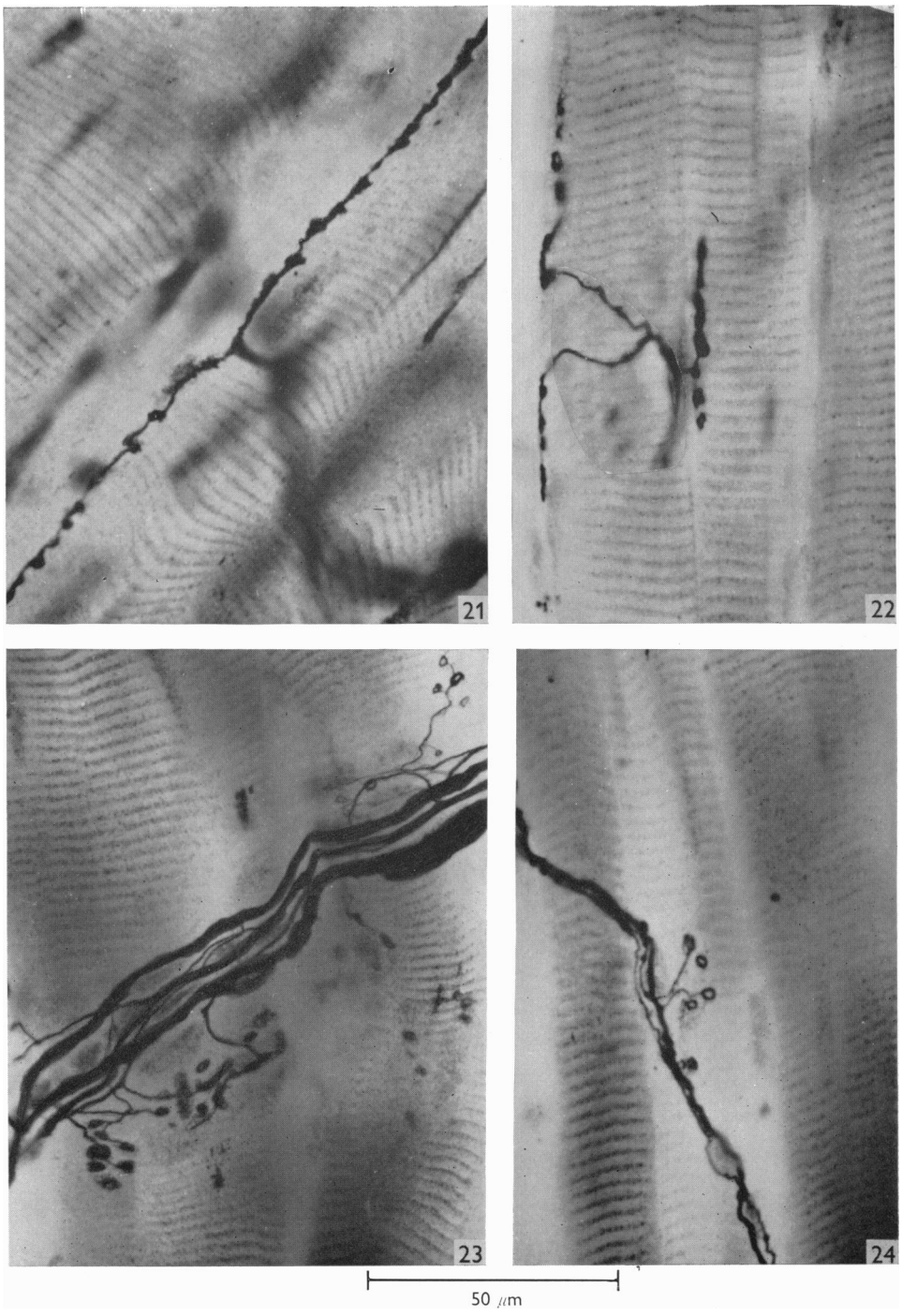
Fig. 20. A graph to show the distribution of nuclei within all the intrafusal fibres of spindle D9, Table 1. The data are plotted in this way to show the slight increase in nuclear density, indicated by the increase in slope of the curve, at the capsular region, which is denoted by the two vertical bars.

intrafusal fibres within the same spindle. In none of the cases was it possible to say that the two fibres formed a complete entity. There was always a faint line between the two fibres, and higher resolution was needed to determine this with certainty.

Skeleto-motor innervation

Two types of extrafusal nerve endings were found:

(*a*) Plate endings: these were found in all muscles stained by the modified de Castro method and in the methylene blue preparations in which motor endings were



Figs. 21–24. Teased silver-stained preparations to show the types of motor innervation. Fig. 12 shows a plate ending on an intrafusal fibre to show the linear array of synaptic contacts along the arms of the T-junction. Fig. 22 shows plate endings on two intrafusal muscle fibres. The endings are shorter than those of plate endings on extrafusal fibres. Fig. 23 shows grape endings on extrafusal muscle fibres. The synaptic contacts tend to lie across the muscle fibre rather than along its axis. Fig. 24 shows grape endings on an intrafusal fibre.

visible. The terminal axon approached the muscle fibre at right angles and formed a T-junction whose arms passed in opposite directions along the surface of the muscle fibre and parallel to its axis (Fig. 21). Synaptic contacts occurred at regular intervals of approximately two to four sarcomeres.

(*b*) Grape endings: these were found only in the muscles stained by the modified de Castro method. Their absence in methylene blue preparations might be attributable to the capriciousness of the method. Grape endings lay across and not along muscle fibres (Fig. 23). Usually each synaptic contact had its own short terminal axon, a branch of the parent terminal axon.

Distinction between plate and grape endings could be made in the majority of cases, but sometimes the appearance was indeterminate, with several terminal branches shorter than those found bearing plate endings and each branch made more synaptic contacts than are found on the branches bearing grape endings.

In muscles which contained both plate and grape endings the plate endings were apparently in the majority but no quantitative assessments of the two were made.

The innervation of the spindle

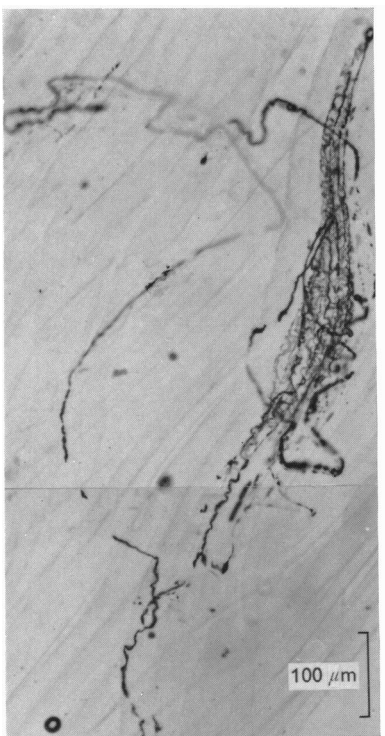
Fusimotor innervation: this was studied in 15 teased methylene blue preparations and in 21 stained by the modified de Castro method. Unless the nerve fibres had been broken during teasing, they could be traced back and were seen to be collaterals of extrafusal motor fibres.

Two types of motor ending were found. Plate endings were similar to those on extrafusal fibres except that the arms of the T-junction tended to be shorter (Figs. 22, 27). Grape endings were very similar to those on the extrafusal fibres (Fig. 24). Eighteen out of 21 spindles stained by the modified de Castro technique contained zones of plate endings, two contained distinct regions of grape endings and 12 contained regions of innervation where grape and plate endings were intermixed. Eight spindles contained plate endings only. All of the spindles had at least one region of fusimotor innervation on each pole. Each methylene blue preparation had plate endings (Fig. 27).

In most cases, the nerve entered the spindle near the regions of synaptic contact, but in one or two cases it entered at the same point as the sensory nerve, and followed a sinuisodal pathway out of the equatorial region to the synaptic contact on the polar region (Fig. 25).

Sensory innervation: each teased spindle contained a single nerve, classified as sensory on the basis of the criteria described on page 522, which formed only one type of sensory ending. The fibre usually divided into two or three branches before entering normally to the spindle axis. Inside the spindle, the branches divided and ramified, innervating each of the intrafusal fibres (Fig. 26, 28). The fine terminals meandered over the surface of the intrafusal fibres and appeared as varicose threads in methylene blue preparations (Fig. 29). These terminations did not form complete spirals around the intrafusal fibres although, as can be seen in Fig. 28, the larger branches sometimes completely encircled one or two muscle fibres. Striations could be observed on all regions of the intrafusal fibres that were supplied with endings.

In some preparations the sensory nerve approached the spindle some distance away from the sensory region, turned through a right angle when it reached the



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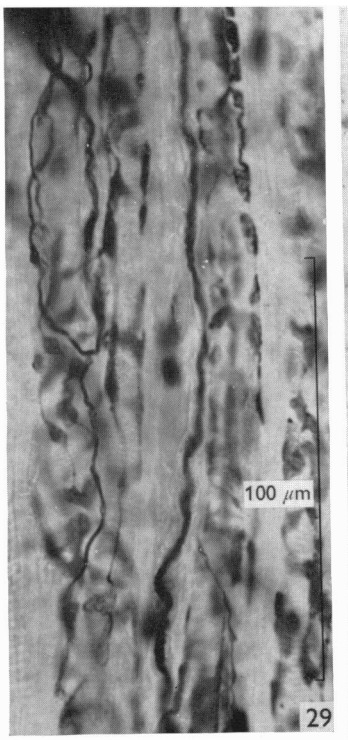

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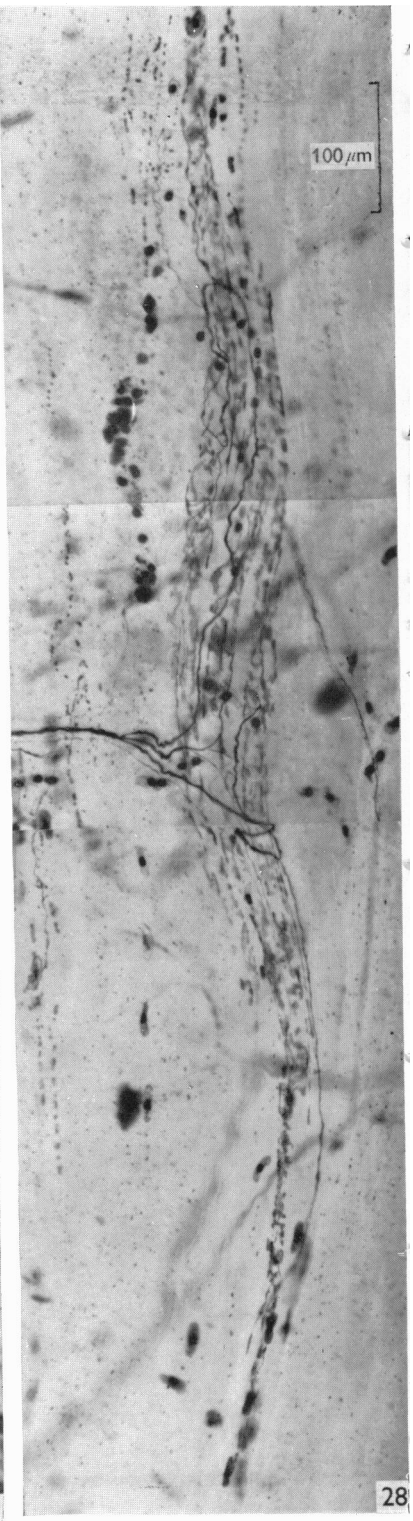

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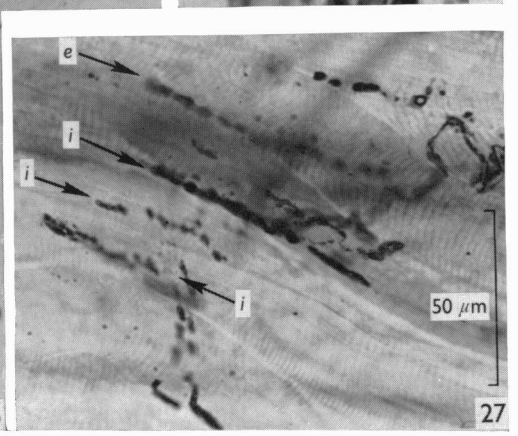

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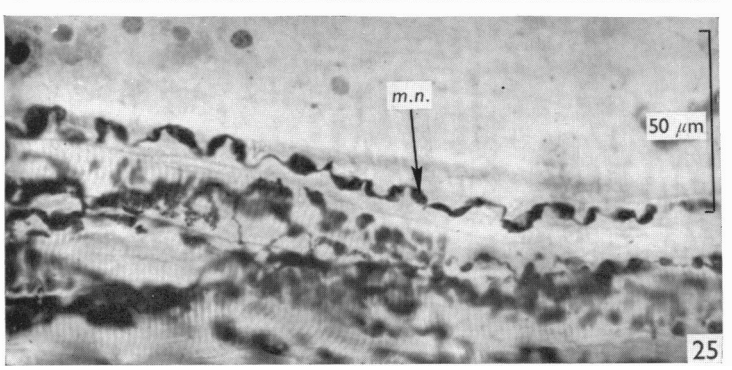

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spindle and passed alongside the intrafusal fibres before branching at the sensory region.

Although each intrafusal fibre has a definite region of sensory innervation, all sensory regions did not always coincide with each other. This is seen in particular to the left of Fig. 28, where a section of the spindle contains just one innervated fibre.

One of the teased preparations (Fig. 26) probably corresponded to the tandem spindles seen in serial sections. Two distinct and separate regions were innervated, but each was supplied by the same sensory nerve.

Measurements of the diameters of undivided sensory nerves of fifty preparations stained by the methylene blue method were made at a distance of 1 mm from the point of entry to the spindle. The distribution was unimodal, with a range of 3·1 to 8·2; the peak lay at 5·5; the mean was 5·2, S.D. 1·08 μm.

DISCUSSION

As observations were made only upon spindles in EDB I muscles of *Testudo graeca* it is therefore impossible to generalize upon chelonian spindles. However, these spindles differed from those of the snake and the lizard in that they were multifibrillar. This supports earlier findings of Huber & de Witt (1897) in *Chrysemys picta* and *Emys meleagris*, in which there were from two to eight intrafusal fibres. The lower figure found in the present study (mean value 10·4) could be due to differences in species as well as muscles.

The mean length of 9·3 mm lies between the value 7·5 mm for spindles of the cat soleus (Boyd, 1962) and of 12·1 mm calculated for six spindles taken from the fourth toe extensor muscle of the frog (Barker & Cope, 1962). It is possible that the length of spindles is related to the size of the muscle, e.g. the smaller muscles C and D (Table 1) have shorter spindles than the larger muscles.

The differences in number of spindles in the four muscles sectioned serially are difficult to explain (cf. B & D, and A & C, Table 1). All the animals were adult and although there is no information on the effect of age on number of spindles in chelonians Cuajunco (1940) showed that in man the number did not change after the 15th week of foetal life. Even though the spindle content of the muscles might be a function of muscle size, number of motor units, or some other parameter, the discrepancy between the two muscles taken from the same animal cannot be explained.

Intrafusal fibres were of one type with an even distribution of nuclei and, apart from variations in diameter in the capsular region, they appeared homogeneous in structure throughout their length. However, the present studies did not include histochemical or electron-microscopical investigations. In the present study the diameters

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Figs. 25–29. Teased preparations to show the pattern of spindle innervation. Figs. 25 and 28 show the sensory regions of two spindles in each of which a single nerve fibre divides and has branches on all the intrafusal fibres. In the spindle of Fig. 26 the nerve fibre has two distinct regions of innervation and the spindle is regarded as a tandem spindle. Fig. 29 shows an enlarged part of the spindle of Fig. 28 to reveal the structure of the terminations of the nerve fibre. Motor nerve endings are shown in Fig. 27; the plate endings on the intrafusal fibres (*i*) and the extrafusal fibres (*e*) appear as rows of dots. Fig. 25 shows a motor nerve (*m.n.*) to the spindle which has entered at the capsular region and passes along the capsular region in a sinusoidal pathway out to the polar regions. (Methylene-blue stain.)

were measured at points midway between the end of the fibre and the edge of the capsule. At these points there were no abrupt changes in the fibre diameter. In comparing the mean value of 10·6 μm for the 282 fibres measured here with those obtained by Boyd (1962) of 30 μm for the cat nuclear-bag fibres and 14 μm for the nuclear-chain fibres, allowance must be made for the fact that Boyd made his measurements at the edge of the lymph space where the fibres are possibly at their thickest. On the other hand, Jahn (1959) obtained average values ranging from 15·3 μm to 21 μm from polar measurements in 19 frog spindles. Further, it is possible that the shrinkage factor of 25% which has been used in the present studies is rather low. It was obtained by comparing the extrafusal fibre diameters in fresh-frozen sections with those in paraffin. No intrafusal fibre diameters were measured and therefore no reasonable estimate can be made of the shrinkage factor of the smallest muscle fibres. Barker & Cope (1962) obtained a value of 45% shrinkage of frog intrafusal fibres in their paraffin preparations.

In the present studies, intrafusal fibres appeared to fuse over very short regions of their length, but verification of this requires electron microscopy, for Corvaja, Marinozzi & Pompeiano (1967) have shown in the cat that light microscopy may be misleading on this point of fusion, although evidence suggests that branching of intrafusal fibres occurs.

The only type of sensory ending found had resemblances to those on lizard and snake monofibrillar spindles with long narrow capsules. There were no spindles corresponding to the monofibrillar spindles with short capsules which give a phasic or dynamic response to mechanical stretch. However, in the absence of electrophysiological studies, functional properties of the tortoise spindle cannot be fully determined, and those in other muscles require investigation.

In contrast to the observations of Huber & Witt (1897), no bulbous fluid-filled capsules were found. The teased preparations are unsuitable for observation of the shape of the capsule but serial sections indicated the structure clearly and the control muscles taken from rat and frog contained spindles with such bulbous capsules. By reference to the electron micrographs in Katz (1961) and also to Figs. 3–6 of the paper by Barker & Cope (1962), it is seen that the tortoise spindle capsule is rather similar to a deflated capsule in a frog where septa enclose individual fibres.

Capsular structure may influence the response to mechanical stretch. Houk, Cornew & Stark (1966) suggest that it may serve to isolate the sensory endings from mechanical disturbances caused by surrounding muscle fibres. Bridgman & Eldred (1964) on the other hand suggest that the fluid-filled capsule may be part of a pressure-sensing device. It would thus seem that the tortoise spindle would be deficient if either or both of these ideas were true. Further, tortoise spindles possess no elastic tissue, which is thought to ensure the rapid return of a stretched spindle to the shortened position (Cooper & Daniel, 1967). Unless there is some other mechanism to achieve this, spindles in the tortoise would not show as rapid a response to the release of mechanical stretch as those of frog and mammal.

Another indication that the tortoise spindle may not be dynamically sensitive is the fact that the sensory ending does not lie upon a structurally different region of the intrafusal muscle fibre. The reticulated zone in the frog spindle and the nuclear bag in the mammalian spindle are thought to be elastic, and detailed mechanical

models have been set up which try to explain the behaviour of such spindles by Houk *et al.* (1966) (for frog spindles) and by Crowe (1968) (for mammalian spindles). However, this speculation is made with caution, for although the tortoise intrafusal fibre does not appear to possess such zones, there is no functional evidence to determine whether or not it exhibits a dynamic response. For example, functional differentiation might reside in myoneural junctions or in the close approximation of the capsular tissue to the fibre to the central region.

The presence of both grape and plate endings upon the poles of the same spindle is unexpected in view of the observations of Szepsenwol (1960) upon the innervation of the lizard *Anolis*. He found that each monofibrillar spindle had either grape endings or plate endings but never both. Further, it is thought that the long-capsulated spindles of snake and lizard have a static/plate collateral motor innervation and that the short-capsulated spindles have a dynamic/grape collateral innervation (Barker, 1968). The tortoise spindles cannot be regarded as corresponding to bundles of the monofibrillar spindles found in lizard or snake because, although the sensory endings may be similar in structure to the long-capsulated spindles, a dual intrafusal motor innervation has not been seen in the lizard or snake.

In many respects the tortoise spindle differs from those of other species and knowledge of its properties must await the outcome of physiological experiments.

SUMMARY

1. The distribution and dimensions of spindles and intrafusal muscle fibres were studied in the ext. dig. brev. I muscle of the adult female tortoise *Testudo graeca*, in serial sections and teased preparations.

2. Only one type of intrafusal muscle fibre was found. Myofibrillar density and nuclear distribution were fairly uniform throughout their length. Fibres appeared to branch and possibly to fuse; diameters were maximal on either side of the capsule.

3. The capsule was not bulbous and contained no large fluid space, but within it each intrafusal fibre had closely approximated sheaths of connective tissue. Further sheaths surrounded the complete spindle, and within this smaller groups of fibres had separate sheaths.

4. Only one type of sensory ending was found and each spindle had only one sensory nerve.

5. The motor innervation was supplied by collaterals of nerves to the extrafusal fibres. Both plate and grape endings were found, but not always on the same spindle.

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