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THE SIZE OF MOTOR UNITS DURING POST-NATAL DEVELOPMENT OF RAT LUMBRICAL MUSCLE

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SUMMARY

1. The number of muscle fibres innervated by individual motor neurones (motor unit size) was measured in lumbrical muscles of rats aged 0–28 days, during the period of elimination of polyneuronal innervation. Motor unit sizes were determined from twitch tension measurements combined with muscle fibre counts made from histological sections of the muscles.

2. The relative tensions contributed by individual motor units declined from about 25 % of the total tension at birth, to about 9 % at 28 days of age. Intracellular recordings showed that part of this decrease reflected the elimination of synapses from polyneuronally innervated muscle fibres.

3. During the same period, however, new muscle fibres were produced. The total number of muscle fibres present increased from about 500 at birth to about 950 fibres in mature muscles.

4. These two processes were offsetting: some synapses were eliminated (from polyneuronally innervated fibres) while simultaneously others were formed *de novo* (on newly produced muscle fibres). Quantitative measurements showed that for the first 10 ten days after birth, there was little change in motor unit size. Thereafter production of new muscle fibres ceased, and motor unit size decreased to the adult level.

5. It is concluded that during early post-natal development, a lumbrical motor neurone maintains a nearly constant number of synapses, but extensively reorganizes its synaptic field, retracting synapses from some muscle fibres, while forming new synapses with other fibres.

INTRODUCTION

The normal development of skeletal muscle involves a series of interactions between motor neurones and muscle fibres. Early in embryogenesis, motor axons grow into the undifferentiated muscle primordium. Instructions are exchanged that affect the subsequent course of maturation of muscle fibres (see Jacobson, 1978) and also the fate of motor neurones—which ones will persist and which ones will die (Cowan, 1973). Functional synapses develop in such a way that, at birth in mammalian muscle, each muscle fibre receives inputs from several different motor neurones. Postnatal maturation involves the elimination of some of these synaptic inputs so that by 2–3 weeks of age, each muscle fibre is innervated by a single motor neurone, and motor unit size has decreased to the adult level (Redfern, 1970; Bagust, Lewis

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& Westerman, 1973; Bennett & Pettigrew, 1974; Brown, Jansen & Van Essen, 1976; Riley, 1977).

The present report concerns a study of post-natal development in rat lumbrical muscle. This preparation is of interest for two reasons. First, as will be shown, its motor innervation is delivered to the muscle by way of two different, anatomically separated peripheral nerves. This is a useful situation for certain experimental manipulations designed to study elimination of polyneuronal innervation. Secondly, the lumbrical muscle is relatively immature at birth, for it contains only about one half the adult number of muscle fibres. Thus, as the present experiments show, postnatal development involves not only elimination of some synaptic inputs, but also the maturation and *de novo* innervation of other muscle fibres. Quantitative estimates of motor size show that for a time these two events (elimination and formation of synaptic inputs) proceed at about the same rate, so that motor unit size changes little during the first 10 days after birth. A preliminary account of this work was given earlier (Betz, Caldwell & Ribchester, 1979).

METHODS

Experiments were carried out on the fourth deep lumbrical muscle which inserts on the fifth digit of the hind foot in Sprague Dawley rats. This lumbrical muscle receives innervation via both the lateral plantar nerve and sural nerve. The sural nerve anastomoses with the lateral plantar nerve at the heel and the muscle is then innervated by a deep branch of the combined nerves.

Muscle tension recordings. Muscles were isolated with the branches of the lateral plantar and the sural nerve intact. The distal tendon was pinned to the bottom of a Sylgard lined dish. The proximal tendon was attached to a transducer (Vernitron Piezoelectric, Bedford, Ohio). Muscles were superfused with a Ringer solution of the following composition (mM): Na^+ 150, K^+ 5, Mg^{2+} 1·0, Ca^{2+} 2·0, Cl^- 147, HCO_3^- 12, SO_4^{2-} 1, D-glucose 11. Solutions were equilibrated by bubbling with 95 % O_2/5 % CO_2. A few drops of phenol red were added per litre of Ringer solution in order to monitor the pH of the solution. Experiments were performed at room temperature. The output of the transducer was monitored differentially using a WPI dual probe electrometer. The output was filtered and amplified using a Tektronix AM 501 amplifier and displayed on a storage oscilloscope screen and on a Gould pen recorder. The transducer was sensitive to applied forces of less than 10 mg and responded linearly up to 5 g. The frequency response of the system was greater than 150 Hz. The resting tension of muscles was adjusted to give a maximal isometric twitch response. Nerves were stimulated using suction electrodes; stimulation was produced by applied voltages of 0·1–10 V amplitude and 50–200 μsec duration. In adults, the number and size of discrete increments in muscle tension (motor units) evoked by graded stimulation to either nerve were recorded. These increments of tension were used to calculate motor unit size. This is a generally accepted procedure (Brown & Ironton, 1978; Thompson & Jansen, 1977). However, Brown & Matthews (1960) showed that in cat muscles series elasticity can give rise to non-linear summation of the twitch tensions of different motor units. Consequently, twitch tension measurements may lead to over-estimates of the size of lowest threshold motor units and under-estimates of highest threshold motor units. This problem could be avoided by measuring tetanic tensions of single motor units (Bagust, Lewis, Luck & Westerman, 1972). This was not feasible in the present experiments since different motor axons often had close excitation thresholds which led to fluctuations in the tetanic tensions. In any event, the mean motor unit size would be the same regardless of the measuring technique used. In neonatal animals (0–15 days) only the lowest threshold motor units in both the lateral plantar and sural nerves were measured. By reversing the polarity of the stimuli applied to the nerves, different motor units could sometimes be recruited first. In best cases it was therefore possible to measure up to four motor units in the neonate. The maximum twitch response to direct muscle stimulation was obtained by applying a suction electrode to the muscle. A slight amount of negative pressure was applied to form a seal at the mouth of the suction electrode.

Supramaximal stimuli (100 V amplitude and about 1 msec duration) were used to evoke muscle responses. Motor unit sizes were expressed as percentages of the total direct tension.

Histology. After recording muscle tension, adult muscles were frozen in isopentane cooled with liquid nitrogen. Muscles were pinned at their resting length. Transverse sections were cut with a cryostat. Sections were stained with haematoxylin and eosin. The numbers of muscle fibres comprising the adult muscles were counted at 200 × magnification using an eyepiece graticule that formed a 10 × 10 maxtrix over the image of the muscle when viewed under the light microscope.

Neonatal muscles were fixed in phosphate buffered glutaraldehyde, post-fixed in osmium tetroxide, processed and embedded in Epon. Transverse, thin sections were taken through the middle of the muscles, mounted on single-hole formvar coated copper grids, stained and viewed with a Zeiss EM 9S electron microscope. Adjacent thick sections were cut and stained with toluidene blue. Muscle fibre counts were made at 400 or 1000 × magnification and with the aid of a camera lucida. In four muscles, groups of muscle fibres counted using light microscopy were located in the adjacent thin sections using the electron microscope. Muscle fibre counts made in those areas were compared using the two methods. Muscle fibres were identified according to criteria in Results.

Intracellular recording. Micro-electrode recordings were made from muscles from rats of different ages, to determine the time course of the elimination of polyneuronal innervation (Brown *et al.* 1976). Cut muscle fibre preparations (Barstad, 1962) were used to abolish twitching of muscles and to abolish muscle fibre action potentials. The lateral plantar and sural nerves were stimulated separately and the applied stimuli were graded so as to recruit synaptic inputs (e.p.p.s) of different thresholds and latencies. Standard intracellular recording techniques were used.

RESULTS

Adult muscles

The sizes of individual motor units supplying lumbrical muscles were estimated from recordings of motor unit twitch tensions and from muscle fibre counts made from transverse histological sections.

Motor unit tensions. Motor axons reached the muscle via either the lateral plantar or the sural nerve. The former provided the major input to the muscle, while the sural nerve provided the minor input. The twitch tension of an individual motor unit, compared to the total direct twitch tension of the muscle, provided a measure of the fraction of fibres innervated by the motor neurone. Fig. 1 shows typical records of twitch tension evoked by graded nerve stimulation. Superimposed oscilloscope records (Fig. 1*A*) and continuous pen recordings (Fig. 1*B*) were obtained. The incremental steps in tension reflected the recruitment of additional motor units as the nerve stimulus strength was increased. In the cases illustrated, there were nine motor axons in the lateral plantar nerve. This procedure was followed with thirty-one normal adult muscles. These muscles received seven to fourteen motor axons (mean ± S.D. = 11·3 ± 1·9). In nineteen of these muscles the individual contributions of the sural nerve and the lateral plantar nerve were measured. The sural nerve provided up to five motor axons (mean ± S.D. = 1·8 ± 1·5) and the lateral plantar nerve provided seven to thirteen (mean ± S.D. = 10·0 ± 1·7). In general, there was an inverse relationship between the number of motor units in either nerve, suggesting that the axons in the two nerves were drawn from a common pool of motor neurones (cf. Thompson & Jansen, 1977).

The average size of motor units in lateral plantar and sural nerves was the same; each contributed about 9 % of the total direct tension. The distribution of tensions of motor units in the two nerves is shown in Fig. 2.

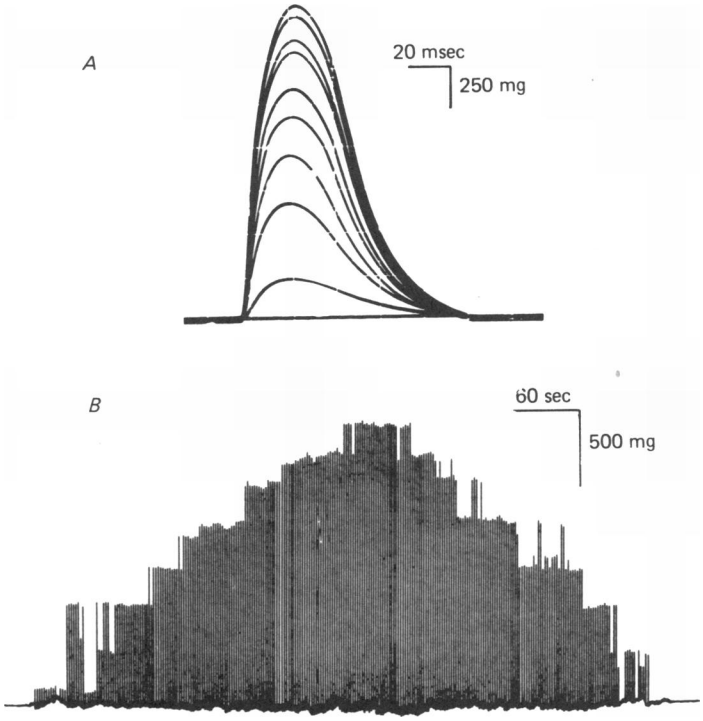

Fig. 1. Tension recordings from adult muscles illustrating the method used to count motor units. *A*, superimposed oscilloscope traces of twitch tensions recorded in response to graded stimulation of the lateral plantar nerve. Nine motor units can be counted. *B*, continuous pen recording of sequential twitch tension responses (from a different muscle). The stimulus strength to this nerve was gradually increased until the maximum response was recorded; then the stimulus was gradually decreased. Nine motor units can be counted in each case, although the order of recruitment of motor units was not exactly the same (cf. the first two motor units recruited).

An additional point concerns the size of the lowest threshold motor unit for each muscle, that is, the first motor unit recruited. The point is important because in neonatal animals (discussed below) the size of only the first motor unit recruited could be measured accurately, due to the overlap of synaptic fields in the young animals. In adults, the tension of the first motor unit recruited in lateral plantar nerve was consistently larger than the over-all average, as has been found in lumbrical muscles of the cat (Bessou, Emonet-Denand & Laporte, 1965). This was not true, however, for the first sural motor units, which generated tensions not significantly different from the over-all average. The explanation for this difference probably lies in the fact that the lateral plantar nerve usually contained about nine motor axons, and the sural nerve only about two motor axons. Thus, assuming that the axons in either nerve are drawn randomly from the pool of motor neurones, the probability that a given axon (say, the one with the lowest threshold) will reside in lateral plantar nerve is 9/11, and for the sural nerve the probability is 2/11. Thus, it is reasonable that the first motor unit recruited in the lateral plantar is larger than the first sural motor unit.

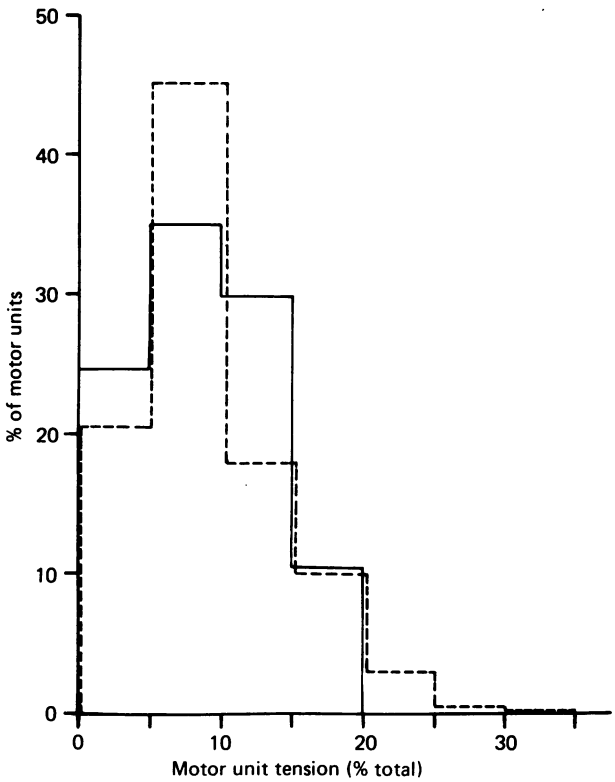

Fig. 2. Distribution of motor unit twitch tensions (expressed as % of total twitch tension) in adult muscles. Motor units from lateral plantar nerve ($n = 263$, dashed line) and sural nerve ($n = 37$, continuous line) are plotted separately; the histograms are very similar, suggesting that motor units from the two nerves are comparable in size.

In summary, the lumbrical muscle received a major portion of its motor supply via the lateral plantar nerve, and a minor portion via the sural nerve. The motor units in each nerve appeared to be drawn randomly from the total population of motor neurones.

Muscle fibre counts. In addition to the tension recordings, the total number of muscle fibres in the adult muscles had to be determined in order to estimate motor unit size. Counts were made from histological sections (see Methods) of the same muscles whose motor unit tensions had been measured. The mean number of fibres in the adult muscles was 938 ± 79 ($\pm$ S.D.).

Motor unit size. The tension and histological measurements were then combined to provide a measure of motor unit size. Specifically, the number of muscle fibres innervated by each motor unit is given by $(t/T) \times n$, where t = single motor unit twitch tension, T = total (direct or indirect) tension, and n = total number of muscle fibres. The mean ($\pm$ S.D.) motor unit size was 87 ± 49. The distribution of motor unit sizes is shown in Fig. 3. The average size of the lowest threshold motor units ($n = 50$) was 105 ± 74 (S.D.).

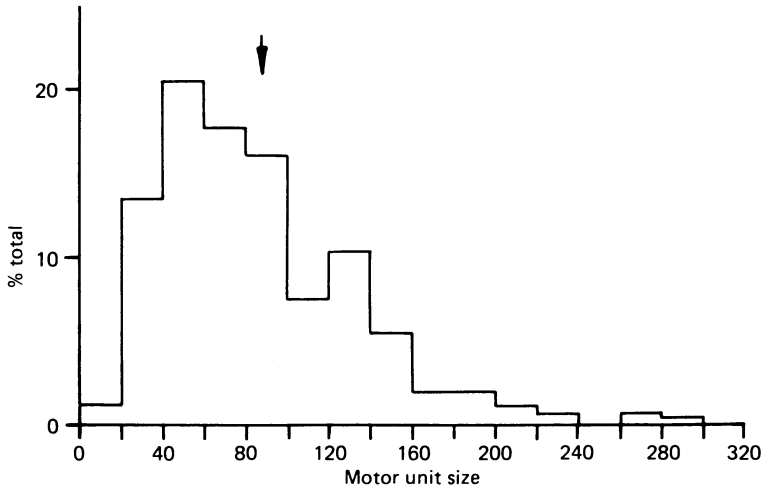

Fig. 3. Distribution of estimated adult motor unit size (number of muscle fibres innervated by a motor neurone). The over-all average motor unit size (arrow) was eighty-seven muscle fibres. The average sizes of sural nerve and lateral plantar nerve motor units were not significantly different (81 ± 66, $n = 32$ and 88 ± 49, $n = 210$ muscle fibres, respectively; $P > 0{\cdot}2$, t test).

Neonatal muscles

The same procedure was used to estimate motor unit size in neonatal rats of different ages (0–15 days), with the exception that only the lowest threshold motor unit sizes were measured. This limitation was due to the fact that synaptic fields overlapped greatly in the young muscles. Thus, except for the first motor unit recruited, measurements of increments in twitch tension would have seriously underestimated the true motor unit tensions.

Motor unit tensions. In new-born (0–2 days) muscles, the first motor unit generated a twitch tension which was about 25 % of the total direct twitch tension (cf. Brown *et al.* 1976). This is considerably greater than in adult muscles (average of first motor units = 11·3 %), indicating that motor units in the neonatal muscle innervated a substantially larger fraction of muscle fibres than did adult motor units. The distribution of motor unit tensions in new-born animals (0–2 days) is shown in Fig. 4. For comparison, the distribution of motor units from mature muscles is also shown (dashed line). The experiment was repeated on animals of different ages, and the results are shown in Fig. 5. Motor unit twitch tension (as per cent of total direct twitch tension) declined progressively over the first 2 weeks after birth.

Muscle fibre counts. The decrease in relative tension of motor units with age could result simply from the elimination of synapses on polyneuronally innervated muscle fibres (Brown *et al.* 1976). However, as is shown below, the situation is more complex in the lumbrical muscle. Muscle fibre counts (of the same muscles studied physiologically) revealed that at birth, only about one half of the adult number of muscle fibres were present.

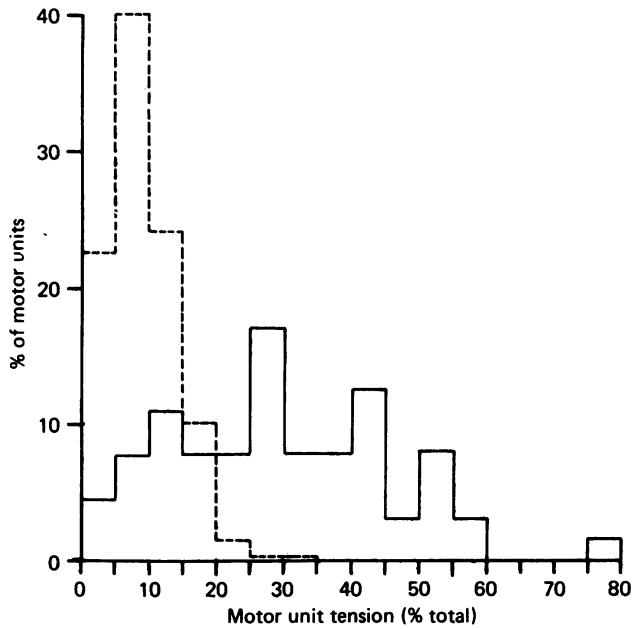

Fig. 4. Distribution of tensions of lowest threshold motor units (as % of total tension) in new-born animals (continuous line). Shown for comparison is the distribution of adult motor unit tensions (dashed line) redrawn from Fig. 2.

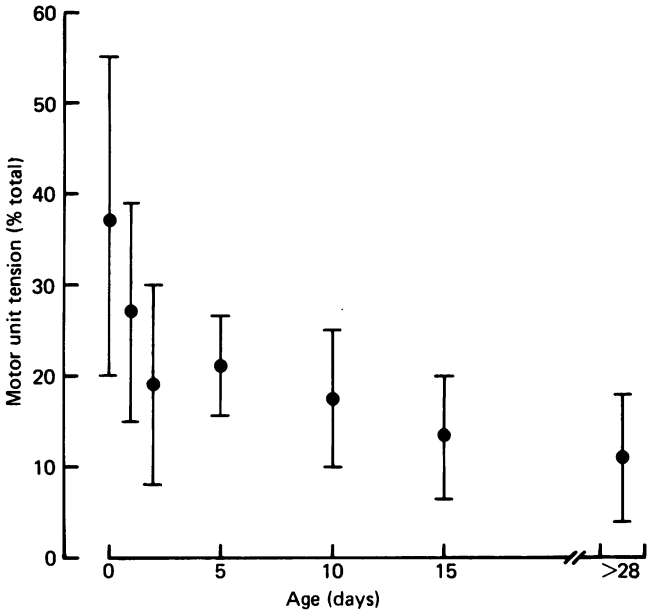

Fig. 5. Time course of reduction in motor unit tension (as % of total tension) during post-natal development. Each time point shows the mean ± S.D. for twelve to twenty-five lowest threshold motor units.

Special procedures were adopted to ensure accurate counts of the muscles from the youngest animals. To be certain that sections were taken only in the mid-belly region, a fine thread was tied around the mid-point of the muscle before embedding, and subsequently identified in the histological sections from which counts were made. There was considerable uncertainty in identifying individual muscle fibres with the light microscope (400–1000 × magnification). In particular, as others have observed (Kelly & Zacks, 1969*a*, *b*; Ontell & Dunn, 1978), closely apposed profiles were not easily distinguished as separate cells. In addition, some small diameter nucleated profiles with smooth contours contained little cytoplasm, and their identity was uncertain. However, even if these were included, the resulting counts were still far below adult levels. Finally, irregularly shaped cells with long, slender processes were easily excluded.

The light microscopic counts were checked in four muscles (from 0–1 day old rats) by examining adjacent thin sections in the electron microscope. Low magnification photo montages were constructed as an aid to subsequent examination at high magnification (94,250 ×). Profiles were carefully examined for the presence of myofilaments and, when cells were closely apposed, for the presence of continuous and separate plasma membranes. Some profiles were of regular shape but contained no identifiable myofilaments. Ontell (1977) showed with serial sections of neonatal rat muscle that such cells often were indeed myogenic. That is, a cell might lack myofilaments in one section and contain them at another level. Such cells probably were myotubes or immature muscle fibres. Accordingly, in the present procedure, cells with regular profiles but lacking identifiable myofilaments were included in the fibre counts. Finally, muscle satellite cells were identified by their close association with muscle fibres and by their heterochromatic nuclei surrounded by scant cytoplasm (Ontell, 1977). These were not counted.

In the four muscles examined in this dual fashion, the electron microscope counts were 20 ± 4 % greater than the corresponding light microscopic counts. Therefore, in the other fifty-nine muscles from 0–2 day old rats, which were examined only by light microscopy, the counts were increased by 20 %. In summary, the procedure insured that all cellular profiles were separately identified, and all cells with myofilaments plus some without (identified as myotubes) were counted. The number present at birth (about 500) was considerably smaller than in the adult (about 950).

The time course of the post-natal addition of muscle fibres was determined by counting muscle fibres in 5, 10 and 15 day old lumbrical muscles. Results are shown in Fig. 6. In muscles from rats aged 5 days or older, there was little or no difficulty discerning discrete muscle fibres, so the light microscopic counts made on those muscles were not altered. This is consistent with results of Ontell & Dunn (1978), who found that small, tightly packed clusters of rat extensor digitorum longus muscle fibres present at birth had dispersed into more easily identifiable profiles by the age of 5 days.

One possible explanation for the reduced muscle fibre counts at birth is that the muscle fibres might not run the entire length of the muscle. Ontell (1977) has shown that myotubes are often only several hundred microns in length. Thus a single transverse histological section might not include all fibres. This is unlikely to be a problem (at least in terms of estimation of those fibres which are innervated) because lumbrical muscles in both adult and new-born animals showed a single band of cholinesterase stain in the middle of the muscle. All of the sectioning for fibre

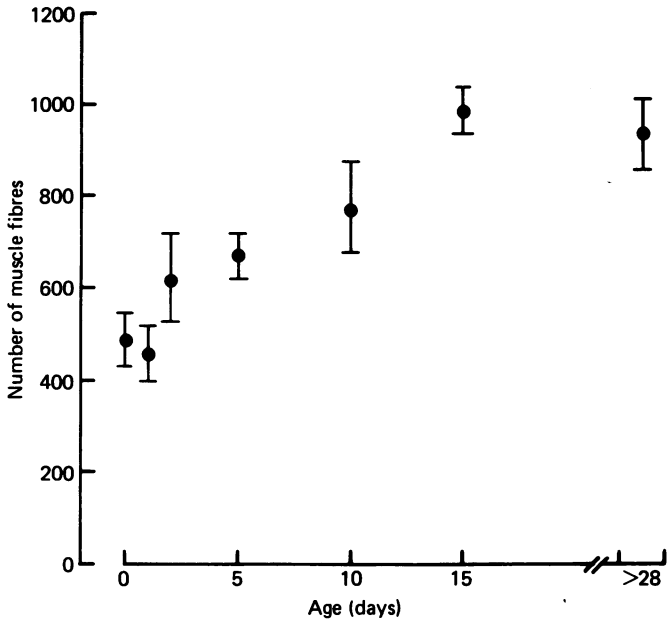

Fig. 6. Time course of development of the total number of muscle fibres. Each point shows the mean ± S.D. for five to ten muscles. Counts of muscles from rats ⩾ 5 days old were made with a light microscope. Counts on younger muscles (0–2 days old) were based on light and electron microscopic observations (see text).

counts was done in this end-plate region of the muscle. It is not known when a myotube becomes innervated, but any myotubes that were innervated should have been counted with this procedure.

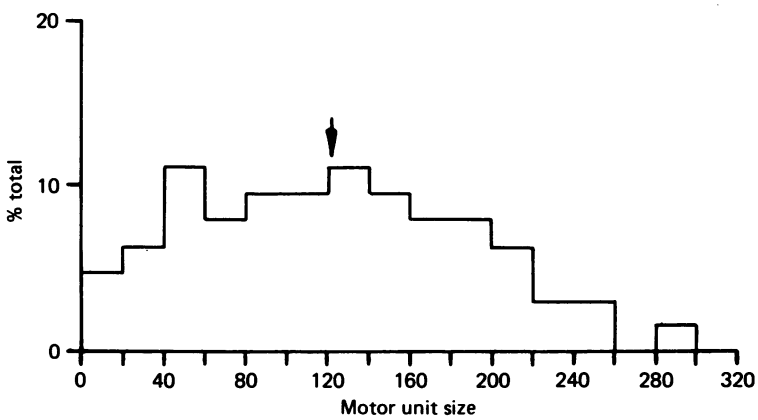

Fig. 7. Distribution of motor unit size in neonatal rats (0–2 days old). The average (arrow) was 122 muscle fibres per motor unit. $n = 63$.

Motor unit size. In muscles from 0–2 day old animals, the mean size of lowest threshold motor units was 145. In adult muscles, the average size of the lowest threshold motor units (105) was about 20 % greater than the average of all adult motor units (87). If this same relationship held in new-born muscles, for which only the lowest threshold motor unit size could be calculated, then the over-all average motor unit size is calculated to be $(145 \times 87/105) = 120$ in 0–2 day old muscles. The distribution of new-born motor unit sizes, each adjusted in this fashion is shown in Fig. 7. It may thus be compared directly with the distribution of all adult motor units, shown in Fig. 3. Evidence that this adjustment is correct is provided in the observation that the average size of lowest threshold *sural* motor units at birth was 123 ($n = 18$). In the adult, sural motor unit size (83) also was about the same as the over-all average (87) for reasons discussed above.

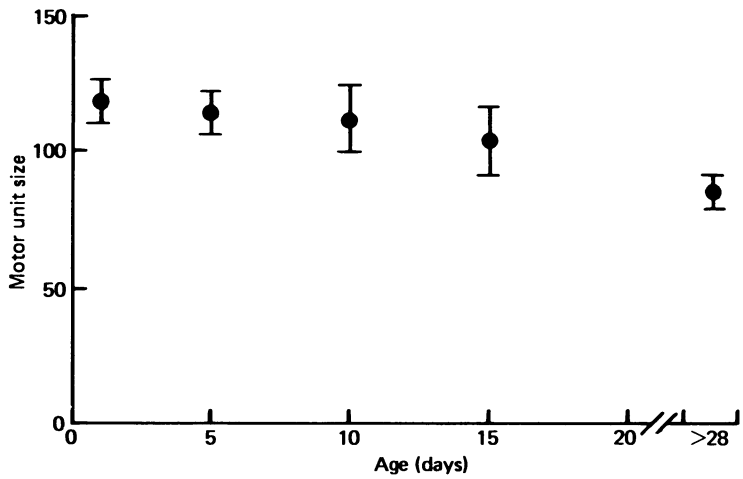

Fig. 8. Estimates of average motor unit (mean ± S.E.) in rats of different ages. Motor unit size remained approximately constant at about 120 fibres for the first 10 days after birth, and thereafter declined to the adult level (about eighty-seven fibres). The first point includes data from animals 0–2 days old.

Similar procedures were used to estimate the size of motor units in animals aged 5 days or older. Results are shown in Fig. 8. Motor unit size did not follow the decline in relative tension of motor units (Fig. 5) because of the offsetting nature of the increase in the total number of muscle fibres (Fig. 6). Motor unit size remained roughly constant up until about 10 days of age and then declined towards adult levels.

In summary, twitch tension measurements showed that new-born motor units innervated about one quarter of the muscle fibres present, and adult motor units only about one tenth. However, muscle fibre counts showed that only about one half of the adult number of muscle fibres were present at birth. Thus the actual number of muscle fibres innervated by a motor neurone (i.e. the motor unit size) at birth was about 40 % greater than the average adult motor unit size. For 10 days or so after birth, motor unit size remained approximately constant, because the elimination of some synapses was balanced by the production and innervation of new

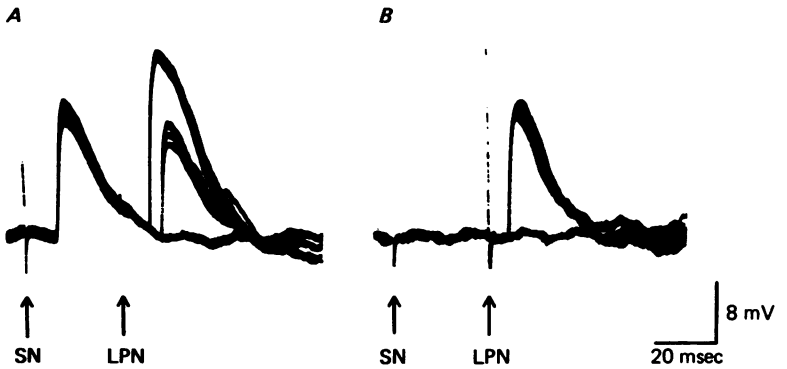

Fig. 9. Superimposed oscilloscope traces of intracellularly recorded end-plate potentials evoked by graded stimulation (arrows) of the lateral plantar (LPN) or sural nerve (SN) in a cut muscle fibre preparation from a 10 day old rat. *A*, this fibre received one synaptic input from the sural nerve and two from the lateral plantar. *B*, this fibre received a single synaptic input from the lateral plantar nerve.

muscle fibres. Thereafter, in older animals, motor unit size decreased to the adult level.

Intracellular recordings. The results described above were used to check the outcome of a wholly independent experiment, namely the time course of elimination of polyneuronal innervation. In this experiment, end-plate potentials (e.p.p.s) evoked by graded nerve stimulation were recorded with intracellular microelectrodes (Redfern, 1970). Both the lateral plantar and the sural nerves were stimulated; typical results are shown in Fig. 9. Inputs from both nerves or incremental steps in the e.p.p. indicated that the fibre was polyneuronally innervated. Twelve to thirty fibres were sampled in each of twenty muscles from animals of various ages. For each muscle, the percent of fibres polyneuronally innervated was calculated. Results are shown in Fig. 10; each filled circle represents data from one muscle. The asterisks are independent predictions (based upon the previously described data) calculated as follows. The total number of synapses in a muscle is given by the product: (motor unit size) × (total number of motor units). The total number of muscle fibres was obtained from histological counts. In neonatal muscles, there were more synapses than muscle fibres, hence at least some muscle fibres were polyneuronally innervated. How the excess synapses were distributed is not known with certainty; here it is assumed that they were distributed in such a way to produce the maximum possible amount of polyneuronal innervation. For instance, in 10 day old muscles, there were about $(113 \times 11{\cdot}4) = 1288$ synapses (113 = average motor unit size in 10 day old muscles; 11·4 = average number of motor units) and about 774 muscle fibres. Thus there were $(1287 - 774) = 514$ excess synapses. If these were distributed evenly, then the muscle could contain at most 514 (= 66·4 %) polyneuronally innervated fibres. Expressed analytically, the predicted maximum percentage of fibres polyneuronally innervated at time t is given by the following:

$$\% \text{ poly} = 100 \left(\frac{11{\cdot}4 S_t}{M_t} - \right)$$

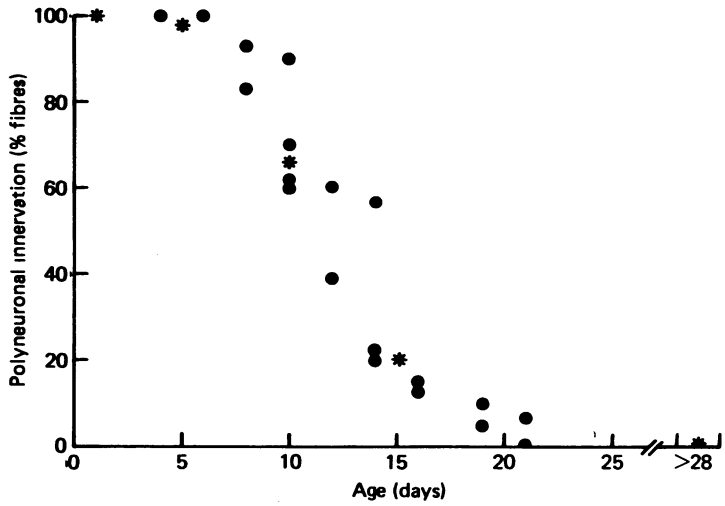

Fig. 10. Time course of elimination of polyneuronal innervation. Each filled circle is from one muscle and shows the per cent of impaled fibres which received more than one synaptic input. The asterisks show the maximum amount of polyneuronal innervation predicted from independent tension and histological data (see text).

where S = motor unit size, M = number of muscle fibres, and 11·4 = number of motor units.

In summary, estimates from tension recordings and histological counts of muscle fibres agreed well with the observed time course of elimination of polyneuronal innervation. The fact that the maximum amount of polyneuronal innervation was observed indicates that muscle fibres produced post-natally became innervated by more than one neurone.

DISCUSSION

The experiments described in this paper were aimed at measuring a single parameter: motor unit size in developing muscle. The technique involved combined twitch tension measurements and histological counts of muscle fibres. While these tests are somewhat indirect, they gave reasonably accurate estimates of motor unit sizes in adult muscles, which consist of a uniform population of easily identifiable singly innervated muscle fibres. In muscles from new-born animals, however, the situation is more complex, for the muscle presents a spectrum of fibres at different developmental stages, and the estimates of motor unit size are accordingly less certain. For instance, the tension measurements are based on the assumptions that muscle fibres behave independently and receive only suprathreshold synaptic inputs. Electrical coupling between muscle fibres or subthreshold e.p.p.s would result in over- or underestimates respectively, of motor unit size. The existence, much less the quantitative extent of such phenomena cannot be estimated with certainty. In addition, histological counts of muscle fibres are more difficult in new-born than in adult muscles. Identification of primary and secondary myofibres (Kelly & Zacks, 1969*a*; Ontell & Dunn, 1978) presents no great problem if examined in the electron

microscope, but myotubes, even in the end-plate region of the muscle, contain no myofilaments in some sections, and some or all of the myotubes may not be innervated (Ontell, 1977). While these myotubes are a minority population, their presence introduces a further uncertainty. These problems diminish soon after birth; in muscles from 5 day old rats, the fibres have separated from each other and are much easier to identify in the light microscope.

The experiments revealed an orderly post-natal development of rat lumbrical muscle. At the cellular level, three events of special importance were identified, namely the elimination of synapses on polyneuronally innervated muscle fibres, the production of new muscle fibres, and the formation of new nerve-muscle contacts.

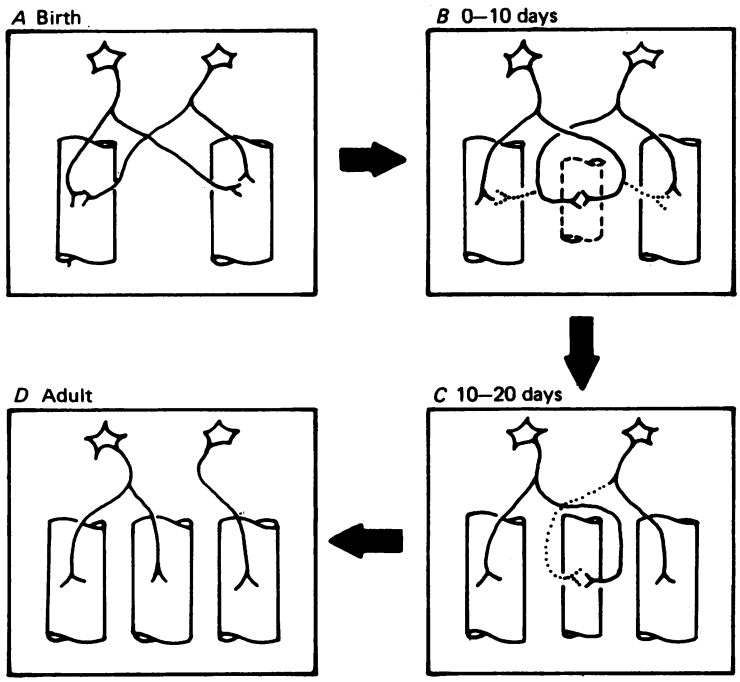

Fig. 11. Schematic diagrams illustrating the post-natal development of rat lumbrical muscle. *A*, at birth all muscle fibres present are polyneuronally innervated. *B*, during the first 10 days after birth motor neurones lose some of their terminals (dotted lines) through synapse elimination, but the same motor neurones form new synapses on newly produced muscle fibres (dashed lines). The new muscle fibres probably become polyneuronally innervated. The two processes (synapse elimination and synapse formation) balance, so that there is little change in the number of synapses made by each motor neurone. Thus, motor unit size remains approximately constant. *C*, between 10 and 20 days of age, muscle fibre production ceases but elimination of synapses from polyneuronally innervated fibres continues (dotted line) and the average motor unit size decreases. *D*, after about 20 days of age, the adult pattern of innervation is reached: each muscle fibre is singly innervated.

The quantitative observations from which this illustration was created (see Figs. 5–8) are as follows: the total number of muscle fibres increased from about 500 at birth to 950 (the adult level) by 15 days of age. The average motor unit size remained at about 120 fibres until 10 days of age, and then decreased to about eighty-five fibres (the adult level).

These results are illustrated diagramatically in Fig. 11. The sequence in which these events occur at the cellular level is unknown. Indeed, none of the six possible sequences (one of which is as follows: synapse elimination→new nerve contact on muscle precursor→maturation of the new muscle fibre) can be ruled out with any certainty. However, the overall rates of the processes were measured. At birth, motor unit size was about 40 % larger than in the adult, and remained fairly constant for about 10 days. During this first 10 days, about 35 % of the total number of adult muscle fibres was produced and innervated. Thus, the relative constancy of motor unit size did not reflect a static pattern of innervation. Rather, a large number of new synapses was formed on the newly produced fibres, and an approximately equal number of synapses was eliminated from other fibres. Moreover, the newly formed fibres apparently became polyneuronally innervated, since no singly innervated fibres were detected (by means of intracellular recordings) in muscles less than 6 days old. At this age about 25 % of the total number of muscle fibres present was produced post-natally. This conclusion is based on the assumption that both newly formed and more mature muscle fibres were sampled with the micro-electrode.

If these events had continued unchanged to maturity, motor unit size would have remained large, and the muscle would have contained more fibres (all singly innervated) than actually observed. Instead, the rate or production of new muscle fibres gradually fell below the rate of synapse elimination, and motor unit size accordingly decreased. The reason for this is not clear. Three possible explanations will be considered. First, motor units may lose the ability to innervate the expanded number of muscle fibres. Such a mechanism of intrinsic withdrawal has been proposed as an explanation of development in rat soleus muscle (Brown *et al.* 1976; Thompson & Jansen, 1977). That is, in soleus, motor neurons are intrinsically 'programmed' to reduce the size of their synaptic fields, and the determination of which synapses are withdrawn is postulated to depend on the degree of competition with other motor neurones at any particular end plate (Brown *et. al.* 1976). Later, of course, motor neurones regain the ability to innervate a larger-than-normal number of muscle fibres, as studies of nerve terminal sprouting in adults have shown (Thompson & Jansen, 1977; Brown & Ironton, 1978; Thompson, 1978).

Alternatively, in the lumbrical muscle, the eventual reduction in motor unit size could reflect a loss of the motor neurons' ability to induce the formation of new muscle fibres. This possibility can be pursued a bit further. The results suggest that the newly formed muscle fibres were polyneuronally innervated. This raises the possibility that the formation of these new muscle fibres *required* the additive efforts of more than one nerve contact. If so, then it is conceivable that in the final stages of synapse elimination, so few new nerve sprouts were produced that no muscle precursors were contacted by more than one nerve. These final abortive efforts would lead to a reduction in motor unit size.

Finally, the reduction of motor unit size might reflect an inability of the muscle to produce additional muscle fibres despite the presence of nerves capable of inducing the formation of and innervating muscle fibres. Such an upper limit on the number of muscle fibres would reflect some property intrinsic to the muscle and independent of the number of motor nerves.

In summary, it is not clear why the final stage of lumbrical muscle maturation

involves a reduction of motor unit size, rather than the production of additional muscle fibres. As discussed, the motor nerves may lose the ability to innervate an expanded number of muscle fibres, or they may lose the ability to induce the formation of new muscle fibres, or the muscle may lose the ability to produce new muscle fibres. These possibilities could be investigated further by measuring the dependence of post-natal muscle fibre production on the number of remaining motor units in partially denervated muscles, an experiment for which the lumbrical is aptly suited, given its dual peripheral nerve supply.

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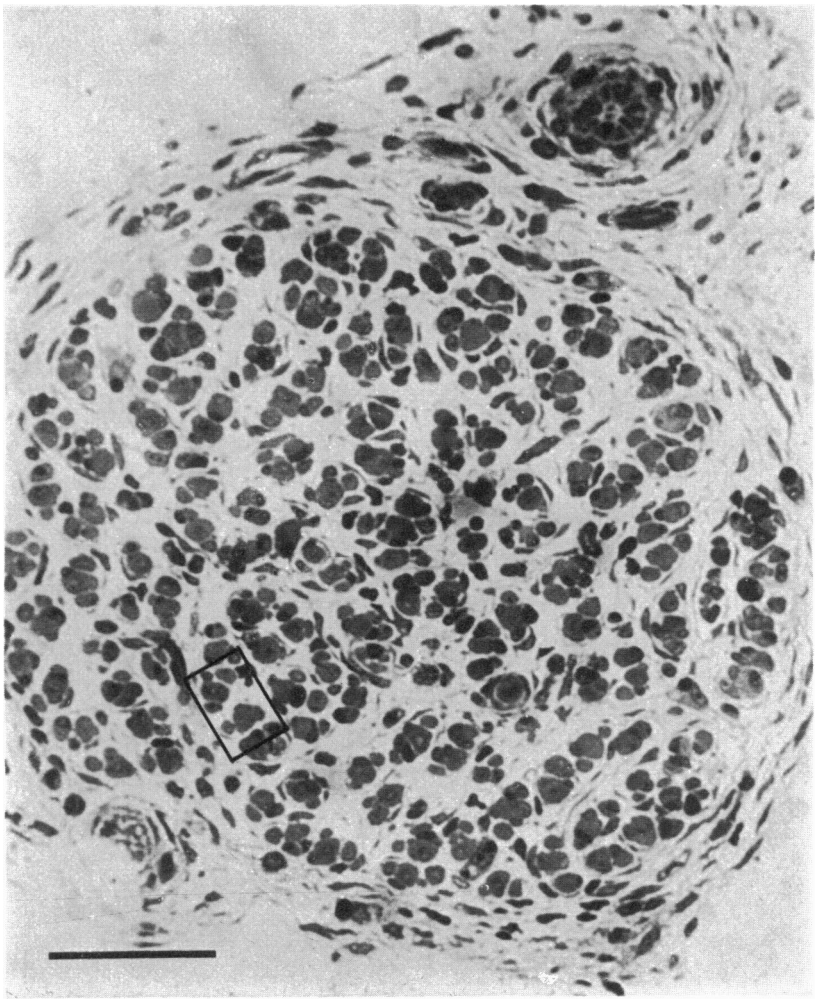
EXPLANATION OF PLATES

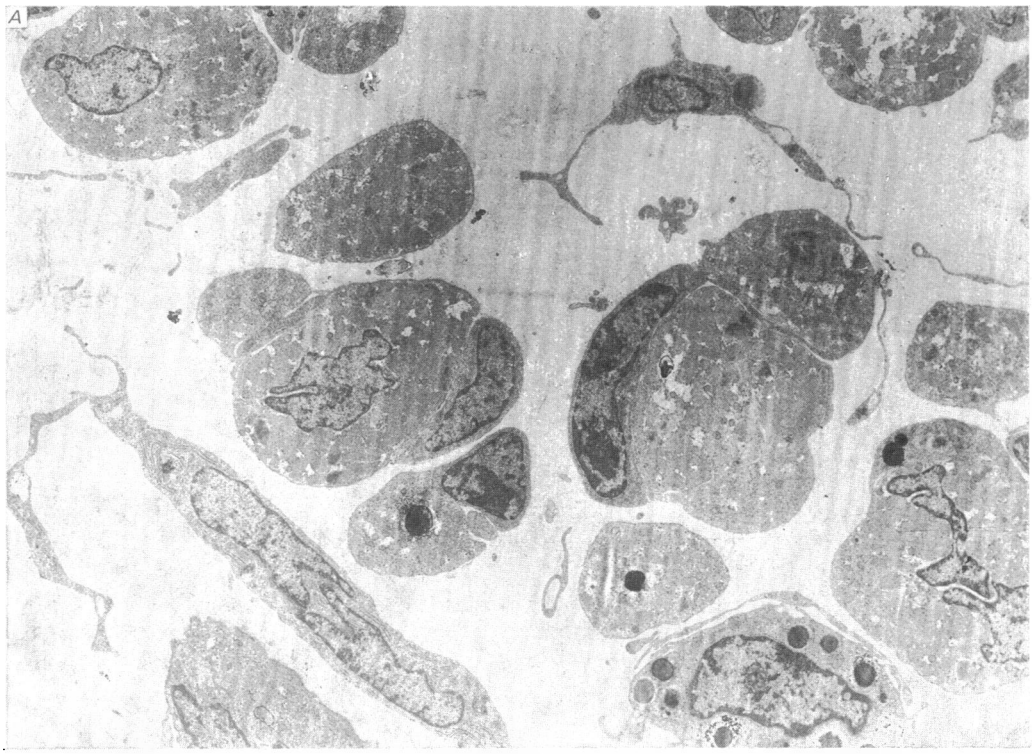
PLATE 1

A light micrograph of a transverse section through the end-plate region of a 1 day old rat lumbrical muscle. The wide range of sizes of cell profiles, combined with the tight packing in clusters, made it difficult to count the total number of fibres accurately. Therefore, adjacent thin sections were examined in the electron microscope. The area marked with a rectangle is shown in Pl. 2. Calibration line = 60 μm.

PLATE 2

Electron micrograph (*A*) of the region of muscle marked in Pl. 1. From a 1 day old rat. Each cell profile was examined (at higher magnification), and the tracing *B* shows the identity of each. F, filamented cell (i.e. cell containing regular arrays of myofilaments); Mt, myotube; SC, satellite cell; Fb, fibroblast; MC, mast cell. In general, criteria proposed by Ontell (1977) were used to identify each cell type. Calibration line = 2·5 μm.

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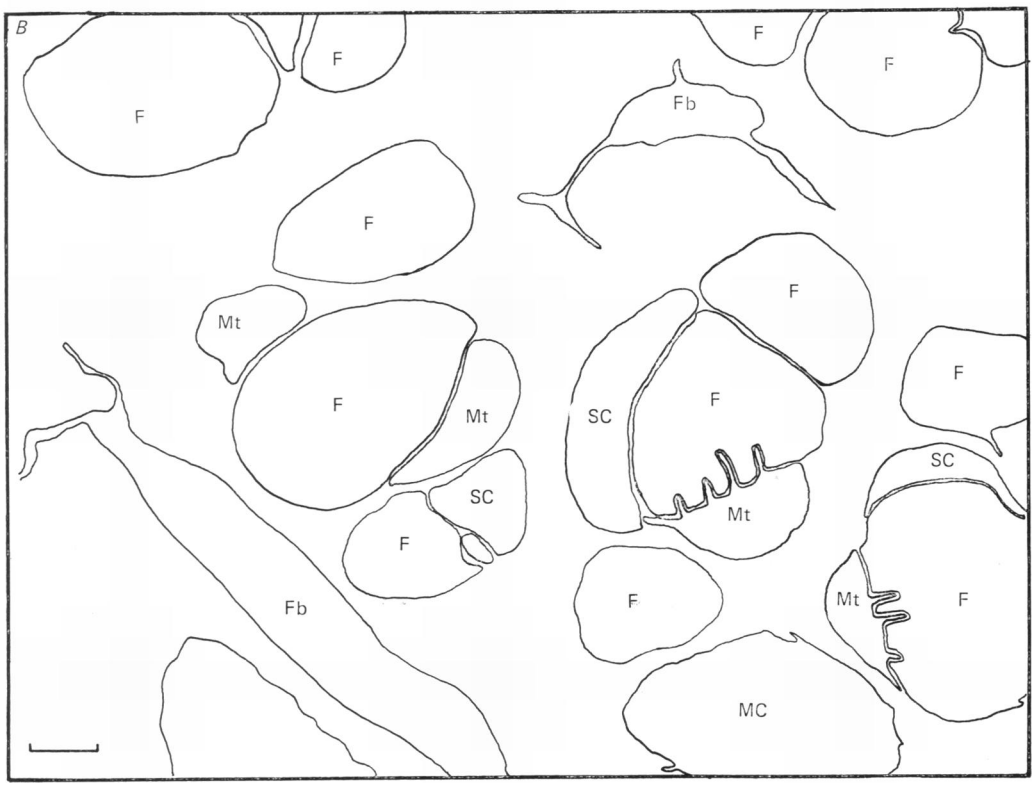
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