*J. Physiol.* (1987), **386**, *pp.* 395–406
*With 4 plates and 3 text-figures*
*Printed in Great Britain*



# RAT MUSCLE DURING POST-NATAL DEVELOPMENT: EVIDENCE IN FAVOUR OF NO INTERCONVERSION BETWEEN FAST- AND SLOW-TWITCH FIBRES

BY S. P. JONES, R. M. A. P. RIDGE AND A. ROWLERSON*

*From the Department of Physiology, University of Bristol Medical School, University Walk, Bristol BS8 1TD and *the Institute of Physiology, The University, Glasgow G12 8QQ*

(*Received 12 June 1986*)

SUMMARY

1. It was confirmed that in the fourth deep lumbrical muscle of the rat the number of muscle fibres at birth is about half that in the adult.

2. The average number of slow-myosin-containing (S) fibres (as determined by specific antibody binding) remains constant from birth to adulthood. Therefore it is likely that all the muscle fibres generated post-natally are type F (i.e. slow-myosin-free).

3. A comparison in the electron microscope between tranverse mid-belly sections from new-born (day 0) and 4 day muscles showed many muscle fibres or myotubes to be intimately associated with other fibres and cells of other types in the new-born, but to be much less closely grouped at 4 days.

4. A full cell count was obtained from electron microscopy of a mid-belly section of a lumbrical muscle at birth.

5. Cross-sectional area measurements in the light microscope at 3–5 days and in the adult showed that at 3–5 days on average the S fibres have a greater cross-sectional area than the F fibres. This is reversed in the adult where the S fibres are the smaller. At 3–5 days the range of cross-sectional areas of F fibres is much wider than for S fibres. Some F fibres are among the largest fibres in the muscle.

6. It is argued from the data that the motor units of adult muscle, which are homogeneous with respect to muscle-fibre types, are produced by selective withdrawal of neonatal motor-unit contacts during developmental synapse elimination.

## INTRODUCTION

A basic question in developmental neurobiology is how correct synaptic contacts are made and, further, how they survive while incorrect contacts are broken. It is now known that in several parts of the developing nervous system there is a period when the number of synaptic inputs to individual cells is greater than it is in the adult condition. Between these two states synapse withdrawal or elimination occurs (for a general review of this topic see Betz, 1986). The question is: what factors decide which synapses persist?

Synapse elimination has been studied most in developing skeletal muscle, following Redfern's (1970) physiological demonstration that new-born rat muscle fibres are

polyneuronally innervated (adult fibres are not). So far two main factors have been implicated in synapse elimination: an intrinsic property of the developing motoneurone to withdraw neuromuscular connexions (Brown, Jansen & Van Essen, 1976; Thompson & Jansen, 1977), and a competitive interaction among presynaptic terminals (Betz, Caldwell & Ribchester, 1980). In this paper we give evidence which leads us to identify a third factor: the recognition of the appropriateness of the post-synaptic element (the muscle fibre) by the presynaptic element (the motoneurone), leading to persistence or withdrawal of the contact.

A preliminary account of some of this work has been given earlier (Jones, Ridge & Rowlerson, 1985).

## METHODS

### *Light microscopy*

Muscles were isolated, frozen, sectioned and stained with periodic acid–Schiff reagent (PAS) or labelled with anti-slow myosin antibody as described in the preceding paper (Jones, Ridge & Rowlerson, 1987). Camera lucida drawings were made and cross-sectional areas measured, also as described previously.

### *Electron microscopy*

The two muscles from a rat at birth (day 0) and from one at 4 days were fixed in 2% (w/v) glutaraldehyde (buffered in 0·1 M-sodium cacodylate at pH 7·2), post-fixed in osmium tetroxide (in the same buffer) under moderate stretch, and embedded in Epon.

They were then sectioned at 50 nm and examined in a Zeiss EM 109 microscope at 80 kV. A low magnification ( × 6642) montage was made of a complete mid-belly section of the muscle at birth. A total cell count was then performed, the cells being divided into identifiable myotubes (containing thick and/or thin filaments), nuclear cells (satellite cells and others cut through the nucleus, and any sections of myotubes where the nuclear profile filled the cell to the exclusion of identifiable myofilaments), morphologically undifferentiated cells, and fibroblasts (rich in Golgi apparatus and rough endoplasmic reticulum). Intrafusal fibres were classified separately where they could be identified by the presence of the spindle capsule, and blood cells and capillary endothelial cells were not counted. The extent of close grouping of muscle fibres and myotubes was compared at the two ages (Pls. 3 and 4).

## RESULTS

### *Number of muscle fibres at different ages*

The numbers of slow myosin-containing (S), slow myosin-free (F) and total fibres in muscles of various ages between birth and adulthood are shown in Fig. 1. The data confirm the finding of Betz, Caldwell & Ribchester (1979) that about half the fibres in the adult are generated post-natally. In addition we find that the number of S fibres remains constant from birth to adulthood (mean number $\pm$ S.D. of S fibres at less than 10 days = 85·3 $\pm$ 7·9 ($n$ = 39); at more than 10 days = 82·1 $\pm$ 6·7 ($n$ = 9)). All the fibres generated post-natally are therefore of the F type, unless there is some closely linked reciprocal conversion of F to S, and S to F types.

The S fibres were identified in mid-belly sections of the muscles by slow-myosin-specific antibody labelling. Examples of such sections cut from muscles at three ages (0, 28 and 86 days) are shown in Pls. 1 and 2. It is striking that in each section the S fibres are distributed roughly evenly throughout the entire section and with no population of intermediate-staining fibres. Polyneuronal innervation would have been high (possibly maximal) at day 0, and synapse elimination (at least in terms

of functional synapses: see Taxt, 1983) would have been complete by 28 days (Betz *et al.* 1979).

Total-fibre counts at birth are impossible to make accurately with the light microscope because of the grouping of myotubes which has been shown by electron microscopy at this age in limb muscles (Ontell, 1977). Betz *et al.* (1979) produced total-fibre counts at this age in this muscle by counting in the light microscope and applying a correction factor (+20%) derived from electron microscopy. We thought it worthwhile to produce a complete count from a mid-belly section by making a montage of electron micrographs covering the entire transverse section.

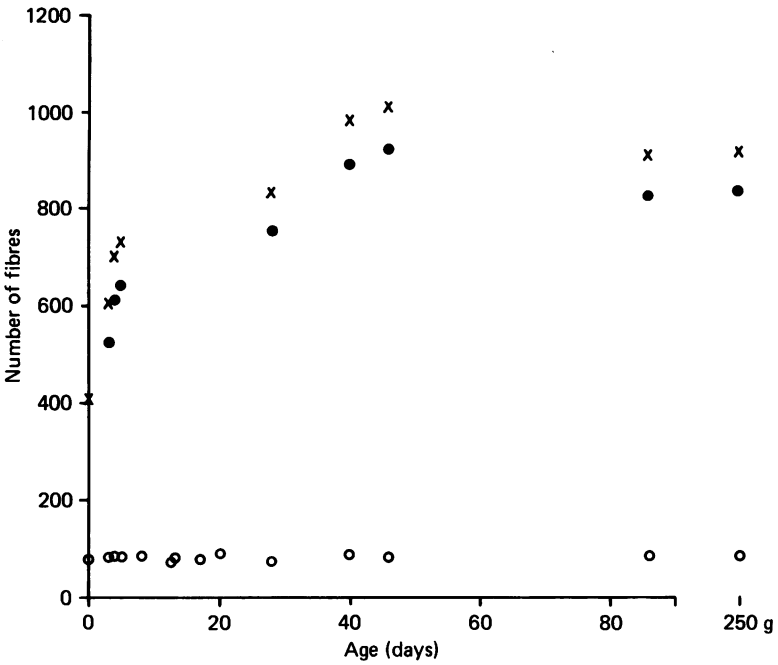



Fig. 1. The number of muscle fibres at different ages in the fourth deep lumbrical muscle. x = total fibres; ● = F fibres; ○ = S fibres.

*Whole-muscle count at birth*

The cells counted were: extrafusal myotubes, 404; intrafusal myotubes (three spindles identified), 4; nuclear cells with the profile of the nucleus, 118; morphologically undifferentiated cells (m.u.c.s; with no nuclear profile) 58; fibroblasts, 88. Capillary endothelial cells, blood cells and all fibroblast profiles not containing a nucleus were not counted. Spindle capsular cells were scored as fibroblasts. Fibroblasts in the epimysium were not counted.

Most muscle cells were *myotubes* with central nuclei (where these were present in the section). They occurred most commonly in small groups consisting of one to four myotubes with one to three nuclear cells which were often closely associated (and sometimes interdigitating) with one or more myotubes. Occasionally small myotubes were similarly arranged in relation to large myotubes. Usually one myotube was larger than the other in the group, as found by Kelly & Zacks (1969) in rat fetal

intercostal muscles, and by Ontell & Dunn (1978) at birth in rat extensor digitorum longus (e.d.l.). Of the classes of cells identified the myotubes were the easiest to identify, the presence of myofilaments being the criterion employed. The count is probably a slight underestimate, as it is possible that some myotubes were sectioned through a portion of their length not containing myofilaments and were classified as 'nuclear cells'. Others may not have extended into the section. However, the total count of myotubes, nuclear cells and m.u.c.s was 584 which is very considerably less than the number of muscle fibres in an adult muscle (our mean value $931 \pm 69$ ($\pm$S.D.) muscle fibres).

*Nuclear cells and m.u.c.s.* These were cells which did not contain recognizable myofilaments or profuse Golgi apparatus. The great majority were closely associated with myotubes. Often they interdigitated with these cells but did not have very widely extended filpodia (as had most fibroblasts). In two thirds of cases a nucleus was present (these were scored as nuclear cells), frequently occupying almost all of the cell profile. This class of cell probably included satellite cells and m.u.c.s (Kelly & Zacks, 1969) cut through the nucleus (these were not distinguishable). A few of the smallest cell profiles without a nucleus (scored as m.u.c.s) could have been the nerve terminals of neuromuscular junctions.

*Fibroblasts.* These cells occurred throughout the section. They are very irregular in shape and small parts of the cells appeared in profusion throughout the section. Consequently only profiles containing nuclei were counted. The count is therefore likely to be an underestimate of the number of individual fibroblasts making an appearance in the section.

*Muscle spindles.* There were three clearly defined spindle capsules in the section. The first contained two myotubes (one much larger than the other), the second contained one myotube and two nuclear cells, and the third contained one myotube and one nuclear cell. Presumably the single (or the larger) myotube would have given rise to a $bag_2$ intrafusal muscle fibre in the adult muscle by analogy with spindle development in the cat (Milburn, 1984). The three large intrafusal fibres were among the largest myotubes in the entire muscle section. It is possible that a few of the myotubes classified as extrafusal were in fact intrafusal, the spindle capsules being at other levels of section in the muscle.

*Myotube grouping at birth and at 4 days*

In the previous paper (Jones, Ridge & Rowlerson, 1987) we report our findings relating to the fibre composition of motor units in the age range 3–5 days, as obtained by glycogen depletion. Our conclusion, that at this age motor units are composed of a mixture of S and F fibres, depends for its validity on the level of electrical coupling between adjacent fibres being fairly low since electrical coupling itself could produce a mixture of glycogen-depleted fibres. By intracellular recording we assessed the level of electrical coupling to be about 12% (Jones & Ridge, 1987). As electrical coupling is likely to be associated with close grouping of fibres (Schmalbruch, 1982) we compared the appearance of grouping at 4 days with that at day 0 (where many gap junctions, which are probable sites of electrical coupling, are known to occur: Schmalbruch, 1982). Representative low power and higher power electron micrographs are shown in Pls. 3 and 4 respectively. It will be seen that close grouping is very marked at day 0 and greatly reduced at 4 days.

*Fibre cross-sectional areas*

If S fibres are generated earlier (perhaps as the primary myotubes: Rubinstein & Kelly, 1981) than F fibres, they might be expected to have larger mean cross-sectional areas than F fibres at early ages. It was not possible to make this comparison with certainty at day 0 because of close grouping of myotubes at this age and the inability of the antibody to distinguish slow myosin in fixed tissue. However at 3–5 days the data support the predictions. In Table 1 are given the mean cross-sectional areas of

TABLE 1. Fibre cross-sectional areas in 3–5 day fourth deep lumbrical muscles. Results are means ± S.D. Figures in parentheses indicate the number of fibres measured

Area ($\mu m^2$)

| S fibres | | F fibres | |
|---|---|---|---|
| PAS +ve | PAS −ve | PAS +ve | PAS −ve |
| 99·7 ± 21·2 (87) | | 66·0 ± 32·4 (604) | |
| 112·5 ± 36·6 (93) | | 59·8 ± 37·1 (612) | |
| 87·5 ± 17·3 (70) | | 52·1 ± 30·5 (524) | |
| 75·5 ± 22·9 (85) | | 45·8 ± 26·5 (524) | |
| 85·2 ± 23·4 (89) | | 48·5 ± 28·5 (635) | |
| 57·6 ± 13·7 (88) | | 35·4 ± 19·5 (676) | |
| 61·3 ± 11·6 (88) | | 35·5 ± 21·9 (592) | |
| 95·9 ± 19·0 (80) | | 60·3 ± 29·0 (656) | |
| 97·1 ± 17·8 (83) | | 58·6 ± 30·1 (676) | |
| 60·0 ± 11·0 (103) | | 35·4 ± 20·6 (607) | |
| 66·5 ± 14·1 (88) | | 43·9 ± 25·4 (641) | |
| 54·1 ± 13·2 (96) | | 36·6 ± 22·6 (658) | |
| 84·1 ± 16·7 (100) | 54·0 ± 24·0 (4) | 55·2 ± 27·9 (644) | 22·9 ± 10·8 (56) |
| 85·2 ± 22·5 (78) | 27·0 (2) | 52·0 ± 34·6 (602) | 15·3 ± 12·6 (62) |
| 92·0 ± 21·0 (88) | 35·5 (2) | 51·7 ± 30·6 (619) | 15·2 ± 9·1 (64) |
| 93·7 ± 19·1 (93) | 90·0 (1) | 60·1 ± 32·3 (615) | 18·8 ± 10·1 (47) |
| 82·6 ± 16·3 (86) | 72·0 (1) | 46·5 ± 26·9 (671) | 17·6 ± 13·3 (68) |
| 79·4 ± 20·7 (96) | 21·7 (3) | 50·6 ± 28·0 (615) | 20·1 ± 13·9 (56) |
| 71·3 ± 20·0 (88) | 38·0 (1) | 45·7 ± 28·3 (641) | 14·0 ± 7·7 (44) |
| 61·6 ± 13·8 (95) | 14·0 (1) | 41·9 ± 22·2 (654) | 14·7 ± 7·0 (52) |
| 97·7 ± 21·2 (87) | | 66·0 ± 32·4 (604) | |
| 107·7 ± 35·8 (80) | | 70·1 ± 37·5 (570) | |
| Mean coefficient of variation: 0·23 | | 0·57 | |

PAS, periodic acid-Schiff reaction for glycogen.

S and F fibres for each of twenty-two muscles in this age range. In all cases the mean cross-sectional area of S fibres is greater than that of the F fibres (mean area of S/F = 1·62, range = 1·47–1·85). Also included in Table 1 are, for eight of the muscles, the cross-sectional areas of S and F fibres that did not stain with PAS and therefore contained little or no glycogen (these muscles had not been stimulated, and so these muscle fibres had not contained glycogen which had been depleted). For S fibres the numbers of such fibres are very small. They may be nuclear bag intrafusal fibres (intrafusal fibres were not included if they were clearly identifiable by the presence of a spindle capsule). In all but two cases they are smaller than the

glycogen-containing S fibres. The numbers and proportions of glycogen-free fibres are much higher in the F-fibre population. Some of these are probably not muscle fibres or myotubes, since it is not possible to differentiate clearly between unstained cell types in the PAS- and antibody-stained sections. In every case the mean cross-sectional areas of the glycogen-free cells are smaller than those of the glycogen-containing fibres.

TABLE 2. Fibre cross-sectional areas in adult fourth deep lumbrical muscles (one muscle at each age). Results are mean ± S.D.

| | Area ($\mu m^2$) | |
|---|---|---|
| Age (days) | S fibres | F fibres |
| 28 | 252·7 ± 54·1 | 355·2 ± 126·8 |
| 40 | 277·3 ± 82·2 | 448·4 ± 158·9 |
| 46 | 325·0 ± 110·2 | 572·6 ± 211·9 |
| 86 | 433·8 ± 102·2 | 794·9 ± 232·7 |
| Mean coefficients of variation: | 0·27 | 0·34 |

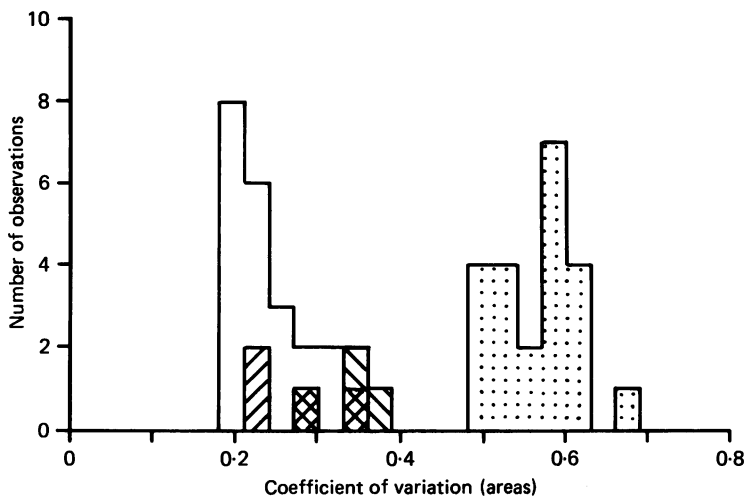



Fig. 2. Distribution of coefficients of variation (with means ± S.D.) for cross-sectional areas of muscle fibres in 3–5 day and adult lumbrical muscles. □ = 3–5 days, S; ▨ = 3–5 days, F; ▨ = adult, S; ▨ = adult, F.

For comparison mean cross-sectional areas for a number of adult muscles are given in Table 2. In every case the mean value for S fibres is smaller than for F fibres (mean area of S/F = 0·61, range = 0·54–0·71, increasing with age). The same thing has been seen in many other fast muscles (e.g. cat gastrocnemius: Burke & Tsairis, 1973).

*The distribution of fibre cross-sectional areas*

If S fibres make their appearance prenatally and roughly synchronously as primary myotubes (Rubinstein & Kelly, 1981), and F fibres are being generated up to about

10 days post-natally, one might expect that at young ages the cross-sectional areas of S fibres would vary less than those of F fibres. Figs. 2 and 3 show this to be the case. For S-fibre cross-sectional areas the coefficient of variation (S.D./mean) ranges from 0·18 to 0·33 (mean = 0·23); for F fibres it ranges from 0·48 to 0·67 (mean = 0·57).

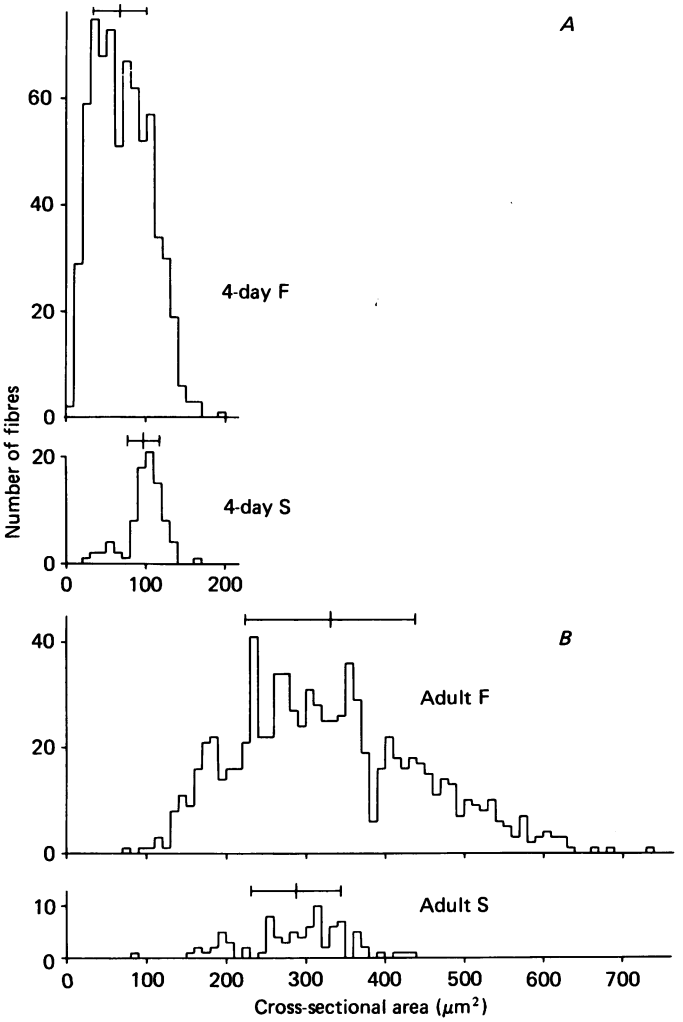



Fig. 3. The distributions of cross-sectional areas (with means ± S.D.) of F and S fibres in a 4 day (*A*) and adult (250 g; *B*) rat lumbrical muscle.

The difference is greatly reduced in the adult muscles, where the mean ratios are 0·27 (range 0·21–0·34) and 0·34 (range 0·29–0·37) for S and F fibres respectively. The distributions of coefficients of variation for S and F fibres in neonatal and adult muscles are shown in Fig. 2. The complete distributions for S and F fibres for two muscles are shown in Fig. 3, a 4-day muscle in *A* and an adult muscle in *B*. (It should be remembered from the results of glycogen staining that some of the smaller profiles in the 'F fibre' population are probably not muscle fibres, and that a few (possibly

drawn from the smaller fibres) of the S fibres are probably intrafusal.) In the 4-day distribution one sees that already a few of the F fibres are the largest in the muscle, and examples can be seen in the high-magnification light micrographs in the previous paper (Jones *et al.* 1987, Pl. 3*B*).

## DISCUSSION

In this paper we have presented evidence that the number of slow-myosin-containing fibres (S) in the fast muscle studied remains constant from birth to adulthood. In addition, we also confirmed the finding of Betz *et al.* (1979) that the total number of fibres almost doubled during the first 2 weeks after birth. Therefore, we conclude that all the muscle fibres appearing after birth are fast (F; that is, they do not contain slow myosin). This conclusion is consistent with our observations on fibre cross-sectional areas. In the adult muscle the S fibres are on average smaller in cross-sectional area than the F fibres; but at 3–5 days the reverse is true, and the range for F fibres is much wider than for S fibres. Many of these F fibres are small, though a minority are among the largest in the muscle. The idea of early development of S fibres is consistent with the findings of Rubinstein & Kelly (1981) in rat e.d.l. muscle which led them to the hypothesis that the primary myotubes in the muscle primordium persisted into adulthood as the small population of S fibres found there.

In the previous paper (Jones *et al.* 1987) we gave evidence that in the lumbrical muscle at 3–5 days the motor units are composed of both F and S fibres, in many cases distributed in a ratio close to that for the whole muscle; that is, many of the motoneurones providing motor supply to this muscle at this age innervate muscle fibres drawn randomly from the total fibre population of the muscle. It is well known that in adult skeletal muscle each motoneurone provides effective innervation to muscle fibres of one type only, as determined by their histochemical profile. The time course of the change from heterogeneity to homogeneity of muscle-fibre type in motor units has not been studied in detail (and here we are concerned only with F and S fibres), but in the absence of evidence of extensive rearrangement of contacts after the period of synapse elimination it is reasonable to suppose that the necessary changes are occurring during the period of synapse elimination.

One can imagine two very different mechanisms that could account for the change from admixture to purity in fibre-type composition of motor units. One would be by fibre conversion under motoneuronal influences, as is well known in adult muscle where the motor supply has been perturbed in some way (e.g. Buller, Eccles & Eccles, 1960: cross innervation; Salmons & Vrbová, 1969: chronic stimulation). In this case at some stage in development the motoneurone would become mature enough to be able to alter any mismatching muscle fibres to match motoneuronal properties. Presumably this would occur after synapse elimination, since before this some muscle fibres would be subjected to conflicting instructions. It is known that such an influence is exerted by motoneurones at a later period in the development of the rat soleus muscle (Kugelberg, 1976) but by then the motor units are probably homogeneous and whole motor units change from fast to slow via an intermediate condition. Thus the known process is not identical to the hypothetical process with which we are concerned here. If such a process explained fully the production of pure

motor units then it would imply that synapse elimination is not directly concerned with this.

An alternative mechanism is that at some stage motoneurones acquire the ability to distinguish between F and S muscle fibres and proceed to withdraw selectively from one or other sort. This could be one of the events associated with synapse elimination, along with other events, such as the known competitive interaction between motor terminals on the same fibre, occurring at the same time (Betz *et al.* 1980; Ridge & Betz, 1984). This could be the component intrinsic to the motoneurones (and independent of competition) described for the developing rat soleus muscle by Brown *et al.* (1976) and Thompson & Jansen (1977). The maturation process could be either in the muscle (the ability to generate an appropriate signal) or in the motoneurone (the ability to perceive the signal) or both.

Our data lead us to favour the second alternative (that motoneurones distinguish between F and S fibres). We give evidence in this paper that the number of S fibres does not change from birth well into adulthood (and well beyond the period of synapse elimination). At the level of visual inspection the distribution of S fibres does not change noticeably throughout this age range (they are fairly evenly scattered throughout mid-belly transverse sections of the muscle); nor do we see large numbers of transforming fibres which would occur if there were type conversion. The simplest explanation of this is that S fibres appear early in development and do not change to F fibres later in the age range. Neither are further S fibres generated post-natally. Any other explanation would require that the opposing forces causing S loss and gain should be equal, which seems unlikely. Since fibre conversion does not take place it follows that some other process (such as selective withdrawal) must be operating to produce pure motor units.

Selective withdrawal during synapse elimination has been implicated also in the development of the topographical projection of nerve supply to some muscles and autonomic ganglia. In some rat and frog muscles individual motor units are selectively distributed across the width of the muscle, the rough position of the fibres in the motor unit being related to the segmental levels of the spinal nerve (Bennett & Lavidis, 1984: rat; Bennett & Lavidis, 1986: frog) and even to the rostrocaudal position in the ventral root (Brown & Booth, 1983: rat) at which the motor axon leaves the spinal cord. This segregation is much clearer after the period of synapse elimination than before, indicating that it is at least partly brought about by selective synapse elimination. A rather similar arrangement is found in the innervation of the superior cervical ganglion of the sympathetic ganglionic chain. Here individual ganglion cells are innervated preferentially by preganglionic axons originating in either more rostral or more caudal thoracic segments of the spinal cord and ascending to the ganglion via the cervical sympathetic trunk. Again this segregation is sharpened during developmental synapse elimination (Lichtman & Purves, 1980: hamster).

These findings and those of the present work lead one to conclude that there must be some recognition of the precise nature of the post-synaptic element by the presynaptic element during the period of developmental synapse elimination. Recognition between pre- and post-synaptic elements can also be inferred from some experiments on regenerating nerve supply following nerve section. For example, in

nerve regeneration into muscle a foreign nerve (previously innervating a different muscle) may form weaker connexions than the sectioned and regenerated natural nerve supply, with a lower quantal content in the end-plate potential (e.g. Holder, Mills & Tonge, 1982: newt), and these may even be displaced by the junctions of the regenerating natural nerve (e.g. Dennis & Yip, 1978: newt). Another example of selective regeneration of nerve axons into muscle is provided by the piriformis muscle of the frog. Here small motor axons regenerate back to tonic muscle fibres, while large axons reinnervate twitch fibres, thereby re-establishing the normal pattern of motor supply (Elizalde, Huerta & Stefani, 1983). In these experiments selective guidance down the previous neural tubes was prevented, and so the result indicates that the regenerating axons recognize the type of the muscle fibre.

In some of these cases it is not known whether the production of selective innervation involves selective synapse withdrawal following initial polyneuronal innervation, or axonal guidance in the formation of the contact. In either case recognition of the post-synaptic element by the presynaptic element must be involved, employing some sort of label. The nature of these labels is largely unknown, although recently Wigston & Sanes (1985) have shown that the label involved in the topographical innervation of superior cervical ganglion cells by segmental axons may be quite general. In their experiments, adult rat intercostal muscles that had been removed from their normal site and implanted into the neck were functionally innervated by axons growing out of the cut cervical trunk (the superior cervical ganglion having been excised). The interesting finding was that for each implanted muscle the innervating axons were selectively drawn from either the more rostral or more caudal thoracic segments corresponding to the more rostral or caudal origin of the muscle. Thus the segmental level of the muscle was recognizable to the innervating preganglionic axon, even though these axons had not previously contacted muscle. Thus each axon was capable of detecting a label (perhaps different densities of the same label) either on their natural post-synaptic contacts in the ganglion, or on intercostal muscles from different segmental levels.

It would be very interesting to identify any of these post-synaptic labels and learn how they are detected by the presynaptic element and how their detection leads to appropriate action by this element.

We thank Miss Sonia Wernberg-Møller and Mrs Debra Hoskins for excellent technical help, and the Wellcome Trust for support. We also thank Drs W. J. Betz, J. H. Caldwell, R. R. Ribchester and D. A. Tonge for reading the manuscript and for many helpful suggestions, Dr D. M. Woolley for making the muscle blocks for electron microscopy, Mr Ian Montgomery for subsequent sectioning and photography, Mrs S. Maskell and Mrs A. Dodds for typing, and Mr P. Robbins for photography. A.R. was supported by a grant from the S.E.R.C. to N. C. Spurway (GR/C/39644); she thanks Action Research for the Crippled Child for support during the period of preparation of the antibody (1977–79).

## REFERENCES

BENNETT, M. R. & LAVIDIS, N. (1984). Segmental motor projections to rat muscles during the loss of polyneuronal innervation. *Developmental Brain Research* **13**, 1–7.

BENNETT, M. R. & LAVIDIS, N. (1986). Topographical projections of segmental nerves to the frog glutaeus muscle during loss of polyneuronal innervation. *Journal of Physiology* **375**, 303–325.

BETZ, W. J. (1987). Motoneuron death and synapse elimination in vertebrates. In *The Neuromuscular Junction*, ed. SALPETER, M. M. New York: Alan R. Liss, Inc. (in the Press).

BETZ, W. J., CALDWELL, J. H. & RIBCHESTER, R. R. (1979). The size of motor units during post-natal development of rat lumbrical muscle. *Journal of Physiology* **297**, 463–478.

BETZ, W. J., CALDWELL, J. H. & RIBCHESTER, R. R. (1980). The effects of partial denervation at birth on the development of muscle fibres and motor units in rat lumbrical muscle. *Journal of Physiology* **303**, 265–279.

BROWN, M. C. & BOOTH, C. M. (1983). Post-natal development of the adult pattern of motor axon distribution in rat muscle. *Nature* **304**, 741–742.

BROWN, M. C., JANSEN, J. K. S. & VAN ESSEN, D. (1976). Polyneuronal innervation of skeletal muscle in new-born rats and its elimination during maturation. *Journal of Physiology* **261**, 387–422.

BULLER, A. J., ECCLES, J. C. & ECCLES, R. M. (1960). Interaction between motoneurones and muscles in respect of the characteristic speeds of their responses. *Journal of Physiology* **150**, 417–439.

BURKE, R. E. & TSAIRIS, P. (1973). Anatomy and innervation ratios in motor units of cat gastrocnemius. *Journal of Physiology* **234**, 749–765.

DENNIS, M. J. & YIP, J. W. (1978). Formation and elimination of foreign synapses on adult salamander muscle. *Journal of Physiology* **274**, 299–310.

ELIZALDE, A., HUERTA, M. & STEFANI, E. (1983). Selective reinnervation of twitch and tonic muscle fibres of the frog. *Journal of Physiology* **340**, 513–524.

HOLDER, H., MILLS, J. & TONGE, D. A. (1982). Selective reinnervation of skeletal muscle in the newt *Triturus cristatus*. *Journal of Physiology* **326**, 371–384.

JONES, S. P. & RIDGE, R. M. A. P. (1987). Motor units in a skeletal muscle of neonatal rat: mechanical properties and weak neuromuscular transmission. *Journal of Physiology* **386**, 355–375.

JONES, S. P., RIDGE, R. M. A. P. & ROWLERSON, A. (1985). The number and size of slow muscle fibres in a fast muscle during post-natal development of the rat. *Journal of Physiology* **360**, 78*P*.

JONES, S. P., RIDGE, R. M. A. P. & ROWLERSON, A. (1987). The non-selective innervation of muscle fibres and mixed composition of motor units in a muscle of neonatal rat. *Journal of Physiology* **386**, 377–394.

KELLY, A. M. & ZACKS, S. I. (1969). The histogenesis of rat intercostal muscle. *Journal of Cell Biology* **42**, 135–153.

KUGELBERG, E. (1976). Adaptive transformation of rat soleus motor units during growth. *Journal of Neurological Sciences* **27**, 269–289.

LICHTMAN, J. W. & PURVES, D. (1980). The elimination of redundant preganglionic innervation to hamster sympathetic ganglion cells in early post-natal life. *Journal of Physiology* **301**, 213–228.

MILBURN, A. (1984). Stages in the development of cat muscle spindles. *Journal of Embryology and Experimental Morphology* **82**, 177–216.

ONTELL, M. A. (1977). Neonatal muscle: an electron microscopic study. *Anatomical Record* **189**, 669–690.

ONTELL, M. A. & DUNN, R. F. (1978). Neonatal muscle growth: a quantitative study. *American Journal of Anatomy* **152**, 539–556.

REDFERN, P. A. (1970). Neuromuscular transmission in new-born rats. *Journal of Physiology* **209**, 701–709.

RIDGE, R. M. A. P. & BETZ, W. J. (1984). The effect of selective, chronic stimulation on motor unit size in developing rat muscle. *Journal of Neuroscience* **4**, 2614–2620.

RUBINSTEIN, N. A. & KELLY, A. M. (1981). Development of muscle fibre specialization in the rat hindlimb. *Journal of Cell Biology* **90**, 128–144.

SALMONS, S. & VRBOVÁ, G. (1969). The influence of activity on some contractile characteristics of mammalian fast and slow muscles. *Journal of Physiology* **201**, 535–549.

SCHMALBRUCH, H. (1982). Skeletal muscle fibres of new-born rats are coupled by gap junctions. *Developmental Biology* **91**, 485–490.

TAXT, T. (1983). Local and systemic effects of tetrodotoxin on the formation and elimination of synapses in reinnervated adult rat muscle. *Journal of Physiology* **340**, 175–194.

THOMPSON, W. & JANSEN, J. K. S. (1977). The extent of sprouting of remaining motor units in partly denervated immature and adult rat soleus muscle. *Neuroscience* **2**, 523–535.

WIGSTON, D. J. & SANES, J. R. (1985). Selective reinnervation of intercostal muscles transplanted from different segmental levels to a common site. *Journal of Neuroscience* **5**, 1208–1221.

## EXPLANATION OF PLATES

### PLATE 1

Mid-belly section from new-born (day 0) rat lumbrical muscle. Frozen section labelled with slow-myosin-specific antibody. Bar = 50 $\mu$m.

### PLATE 2

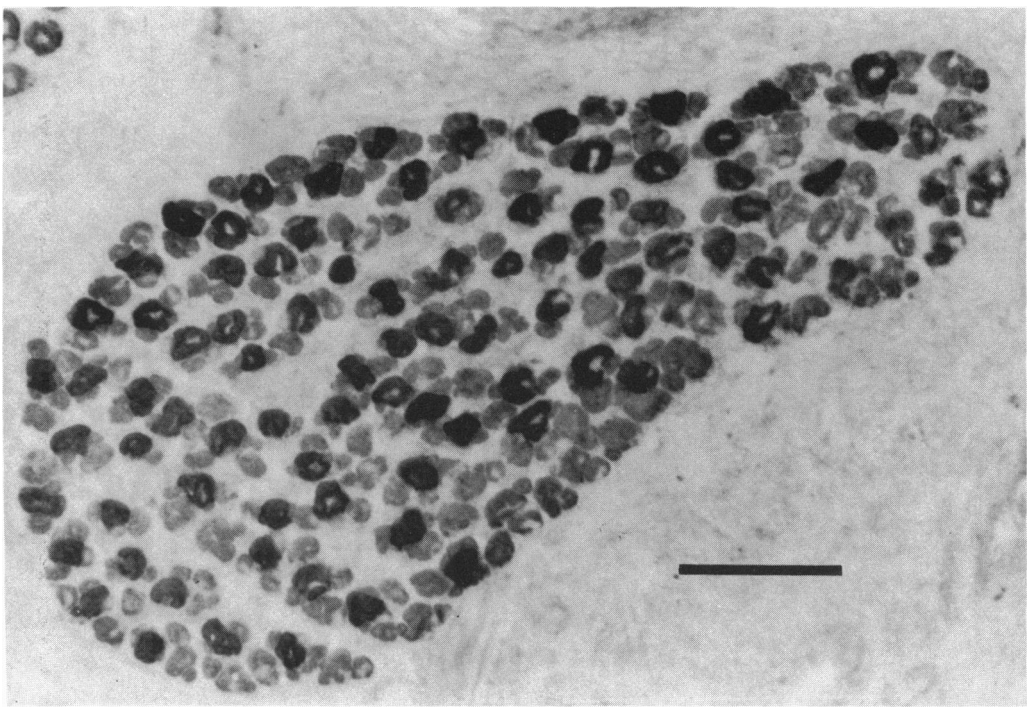
Mid-belly sections from 28 day (*A*) and 86 day (*B*) rat lumbrical muscles (at the same magnification). Frozen sections labelled with slow-myosin-specific antibody. Bar = 200 $\mu$m.

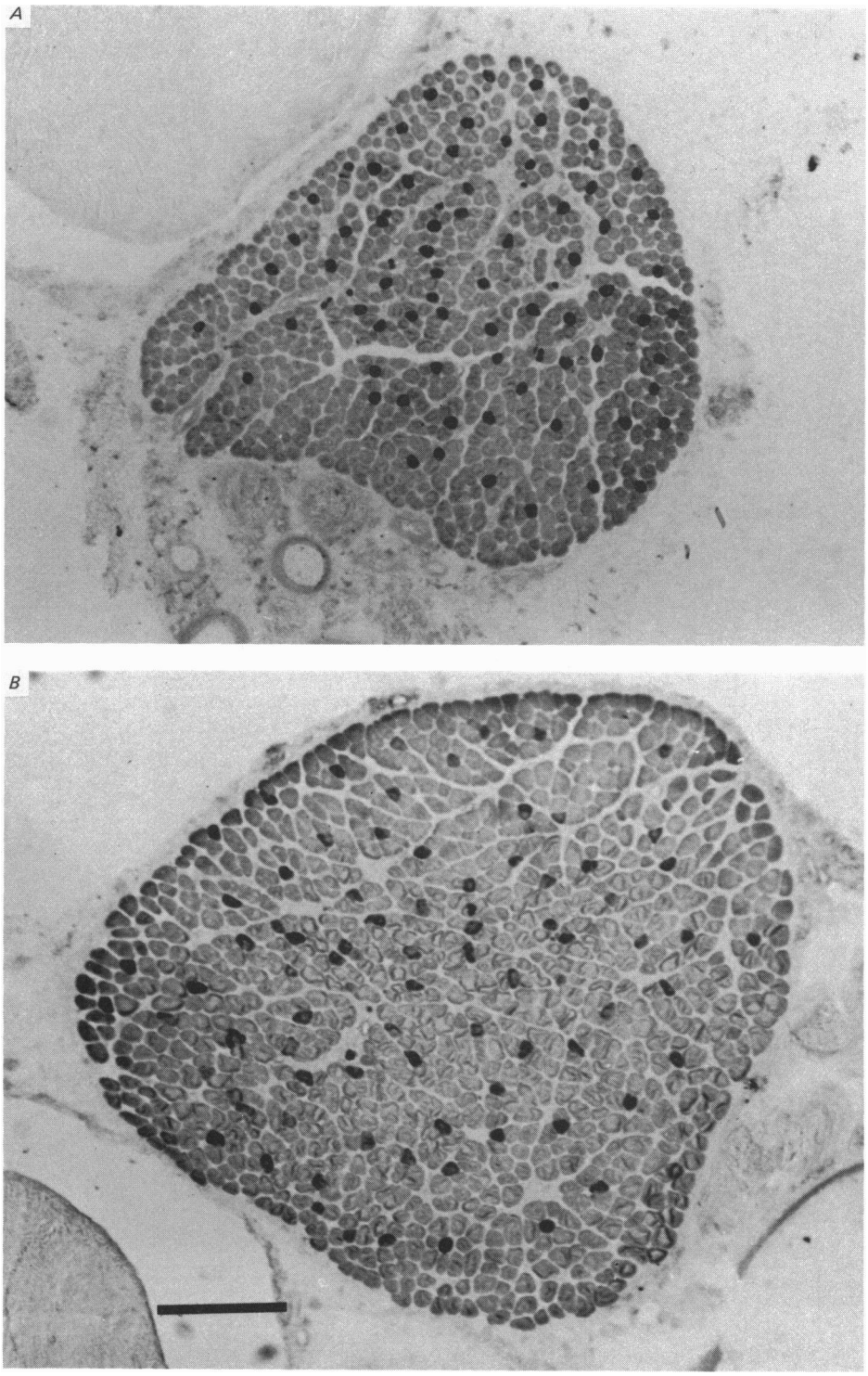
### PLATE 3

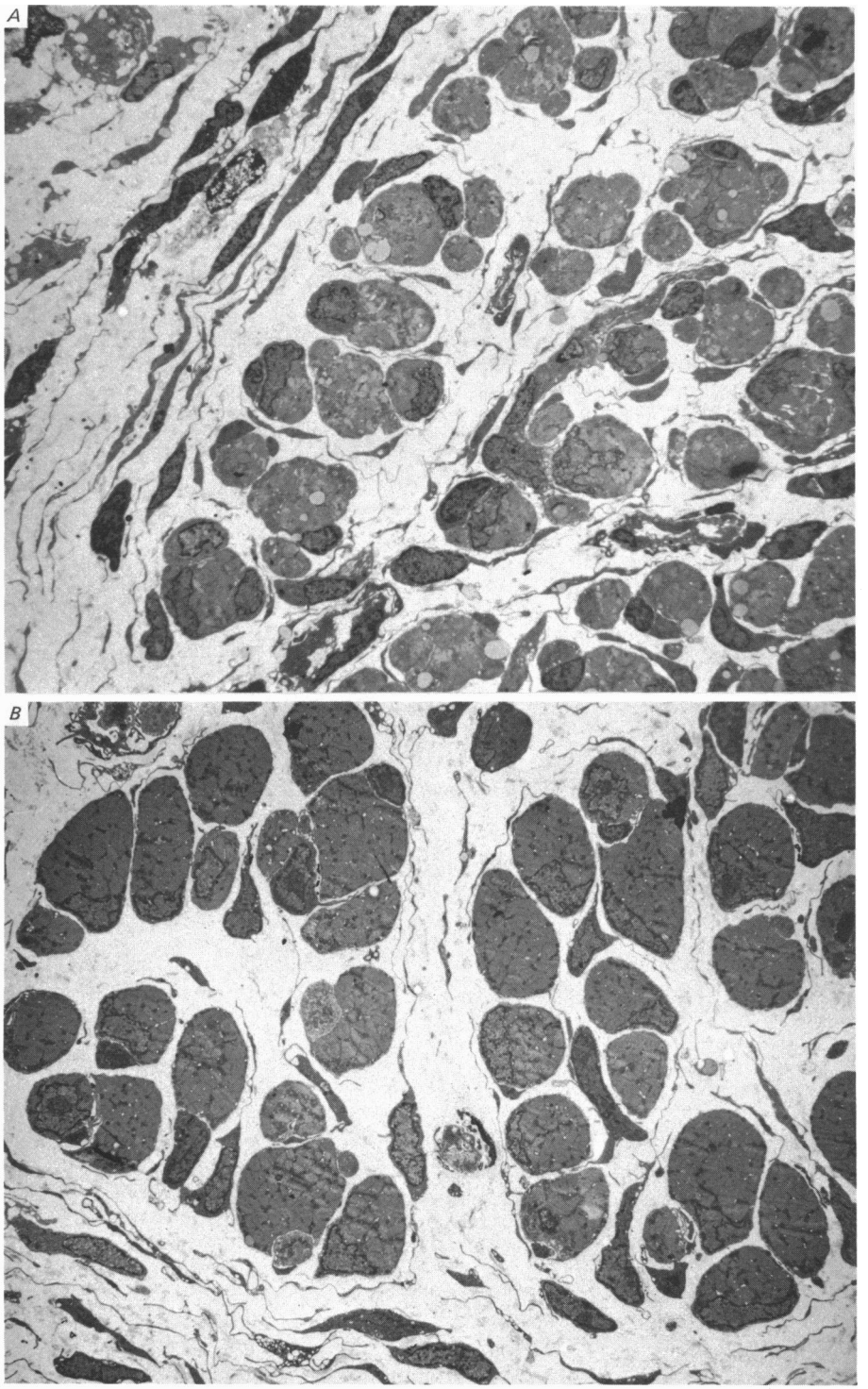
Electron micrographs of transverse sections cut from rat lumbrical muscles at birth (*A*) and at 4 days (*B*). Note the extensive close grouping of cells at birth and their dispersal by 4 days. Bar = 20 $\mu$m.

### PLATE 4

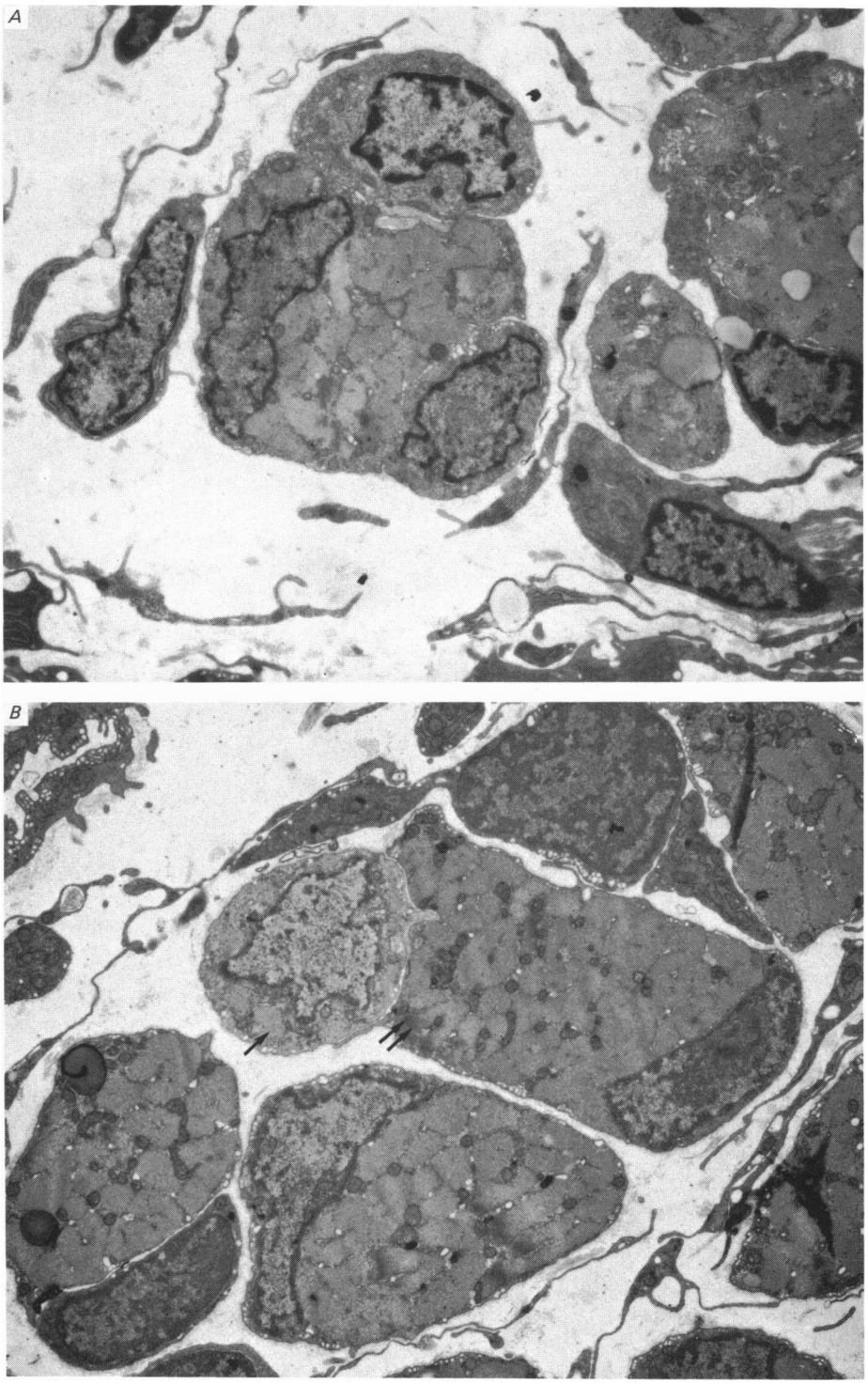
High magnification electron micrographs of transverse sections cut from rat lumbrical muscles at birth (*A*) and at 4 days (*B*). In (*B*) the field has been selected to show an immature myotube (single arrow) in close contact with a muscle fibre (double arrow; myotubes are being generated at this age). Two similar fibres occur in *A* where there are about thirty-five complete muscle-fibre profiles. Bar = 5 $\mu$m.




A
B




S. P. JONES, R. M. A. P. RIDGE AND A. ROWLERSON




S. P. JONES, R. M. A. P. RIDGE AND A. ROWLERSON