J. Physiol. (1984), **356**, *pp.* 303–314
With 1 plate and 5 text-figures
Printed in Great Britain

MUSCLE SIZE AND MOTOR UNIT SURVIVAL IN MICE

BY M. D. HABGOOD, W. G. HOPKINS AND J. R. SLACK
From the Department of Physiology, University of Auckland, Auckland 1, New Zealand

(*Received 6 March 1984*)

SUMMARY

1. The soleus muscles in neonatal and adult mice were surgically reduced in size on one side of the animal.

2. The experimental and control muscles were excised 6–48 weeks later and the number of motor units in each muscle was estimated by stimulating the muscle nerve and counting step increments in the electromyogram recorded *in vitro*. Multiple innervation in individual muscle fibres was then assessed by intracellular recording and by visualization of end-plates in the light microscope with cholinesterase stain. Muscle fibres were counted in cross-sections of each muscle in the light microscope.

3. Surgical reductions in the size of the muscle during the first 3 weeks of life produced correlated reductions in the number of motor units in the muscle. This could not be attributed to masking of motor units by multiple innervation, which was always less than 10% in these muscles.

4. The loss of motor units was greatest following reduction in muscle size in newborn mice, whereas in 6-week-old mice there was no significant loss of motor units following the operation. Thus, survival of neonatal motor units shows an age-related dependence on the number of muscle fibres available for innervation.

5. In control muscles there was a highly significant correlation between motor unit and muscle fibre numbers, which is consistent with the hypothesis that motor neurone survival during the embryonic period of cell death is dependent upon the number of muscle fibres available for innervation.

INTRODUCTION

During normal development many types of neurone are subjected to a period of cell death that can remove a substantial proportion of the newly generated cells (see review by Hamburger & Oppenheim, 1982). Early findings indicated a key role for the target tissue in cell death: removal of chick limb buds caused the complete loss of the sensory and motor nerve cells that were deprived of their targets (Hamburger & Levi-Montalcini, 1949; Hamburger, 1958). In normal embryos, the loss of cells at spinal levels innervating the limbs was found to be less extensive than at levels innervating the trunk and neck regions, where there is less tissue to be innervated (Hamburger, 1975); and extra limb buds grafted onto the trunk became innervated and thereby produced less neuronal death at the spinal levels innervating the grafts (Hollyday & Hamburger, 1976). These and other observations led to the hypothesis

that cell death could be a means of matching appropriately the sizes of independently generated pre- and post-synaptic cell populations by limiting the survival of the presynaptic cells (e.g. Cowan, 1973). It is generally thought that this matching is effected via a competition between the neurones for some agent that promotes their survival and that is available in limited quantity in the target (e.g. Purves, 1980).

A simple test of the competition hypothesis is to examine the effect of varying the relative sizes of neurone and target cell populations. For example, increasing the relative size of a pre-cell-death neurone population should produce a greater degree of cell death as a result of increased competition for the target. Lamb (1980) recently succeeded in doubling the size of the neuronal populations innervating the tadpole limb by diverting the outgrowing nerves on one side of the animal into the contralateral limb. Surprisingly, there was no extra death of motor neurones on either side of the cord, and it was therefore argued that mechanisms other than a competition-based matching of neurone and target population sizes might be responsible for cell death.

An alternative means of achieving neurone and target mismatch would be to vary the size of the target rather than the size of the neuronal population. Laing (1982) attempted to do this by performing partial limb bud extirpations in chick embryos before the period of cell death. He noted good correlations between the resulting size of the limb and the number of surviving motor neurones, which appears to support the competition model. However, the operations led to the complete absence of some limb muscles rather than to a reduction in their size. Since axons in chick limbs appear to be guided accurately to their target muscles (Landmesser, 1980), the complete loss of a muscle would be expected to lead to the death of all its neurones in the same way that complete limb extirpation produces complete neuronal death. Hence, Laing's observation of a correlation between limb size and neurone survival neither supports nor refutes the competition hypothesis and it is not inconsistent with Lamb's observations on tadpoles.

A more critical test of the competition hypothesis would be given by an analysis of the effects on motor neurone survival of varying the size of a specific muscle. Laing's work shows that this would be difficult to achieve in embryonic animals. However, it is known that neurones innervating the limbs of neonatal rodents are dependent for their survival on contact with their targets in the limb (Hall & Schneiderhan, 1945; Romanes, 1946), and the period of motor neurone death in rodents may even extend into the first few weeks of neonatal life (Romanes, 1946; Rootman, Tatton & Hay, 1981; Bennett, McGrath, Davey & Hutchinson, 1983; but see Brown, Jansen & Van Essen, 1976; Lance-Jones, 1982). We therefore decided to investigate whether post-natal surgical reductions in the size of a mouse muscle would produce a correlated reduction in the survival of motor neurones. The reductions were carried out at various post-natal ages, and motor neurone survival was assayed as motor units innervating the muscle at a later adult stage. The numbers of muscle fibres and motor units in adult control muscles were also assayed, since a relation between these variables might be established at the time of cell death in normal animals. Significant correlations were found between the numbers of motor neurones and muscle fibres in the normal and experimental muscles, which supports the notion that neuronal cell death is determined by some limitation in size or availability of the target cell population for innervation.

METHODS

Surgery

Male and female mice of the Charles River CD-1 strain were used. At ages ranging from 1 to 44 days, the animals were anaesthetized with a mixture of 3% halothane–97% oxygen. The soleus muscle on one side was exposed and a variable proportion of the muscle fibres was stripped out along the entire length of the muscle on the side away from the point of nerve entry. The soleus nerve was also crushed with fine forceps at its point of entry into the muscle. The wound was closed and animals were maintained normally until sacrifice 6–48 weeks later.

Assessment of motor units

Animals were killed by cervical dislocation, and the soleus muscle and its attached nerve were dissected free from both sides of the animal. The muscle nerve was dissected back to the level of the thigh to include part of the sciatic nerve. The preparations were mounted in a bath containing an oxygenated HEPES-buffered Krebs solution, the sciatic nerve was taken up into a suction electrode, and the number of motor units innervating each muscle was assessed by applying a graded stimulus to the nerve and counting step increments in the electromyogram (e.m.g.) recorded with an extracellular electrode (Slack & Hopkins, 1982).

The extracellular electrode was a second suction electrode filled with Krebs solution past the level of the internal silver wire electrode. The tip of the suction electrode consisted of a piece of polyethylene tubing drawn to an internal diameter of approximately 0·5 mm. The tip was positioned vertically directly over the centre of the muscle, which was pinned out stretched over a small ($8 \times 8 \times 2$ mm thick) Sylgard plinth. The magnitude of the e.m.g. was increased by removal of the Krebs solution to the level of the top of the plinth. The muscle remained moist and in contact with the recording electrode through a thin film of solution held in place by surface tension.

It was difficult to obtain consistent reproducible step increases in the e.m.g. if recordings were made immediately after the preparation was dissected, because of the phenomenon of 'alternation' of stimulation of units with similar thresholds (Harris & Wilson, 1971). We found that problems with alternation disappeared and motor unit counts became reproducible if preparations were assayed several hours after dissection, probably because axon stimulation thresholds became more separated. The Krebs solution was changed every 30–60 min between dissection and assay.

The e.m.g. was recorded on a Tektronix oscilloscope with a storage display screen. Step changes in the amplitude at the peak of the e.m.g. as the stimulus to the nerve was increased were scored as individual motor units (Pl. 1*C*, *D*). Occasionally, an additional small unit produced only a broadening of the cumulative trace on the storage screen, but this could be seen at the time of recording as a consistent increase in the e.m.g. amplitude. The stimulus was gradually increased until no new units were recruited. The screen was then cleared, the stimulus reduced to zero and the counting procedure repeated until a consistent unit count was obtained (usually only one repeat of the procedure was necessary).

Assessment of multiple innervation

Following assessment of the number of motor units, *d*-tubocurarine chloride was added to the recording bath (final concentration 0·5–2·0 μg/ml) to reduce end-plate potentials to subthreshold levels. Muscle fibres were then penetrated with a micro-electrode in the end-plate region and repetitive supramaximal and graded stimuli were applied to the nerve. Step increments in the end-plate potential in response to the graded stimulus were scored as functional multiple inputs (Brown *et al.* 1976). Thirty to forty fibres in each muscle were assessed.

Histology

Muscles were fixed in a phosphate-buffered paraformaldehyde solution. Some of the muscles were cut in half anterior to the point where the soleus nerve enters the muscle. The posterior half of the muscle, which contained the end-plate region, was teased into fine bundles of muscle fibres and was stained for end-plate cholinesterase (Karnovsky & Roots, 1964). The fibres were then cleared, mounted, viewed in the light microscope and scored for the presence of one or more than one discrete patch of cholinesterase reaction product. Twenty to forty fibres in each muscle were examined.

The anterior halves of all muscles were embedded in Epon and the number of muscle fibres in each muscle was counted in cross-sections (0·5 μm thick) in the light microscope (Pl. 1*A*, *B*).

Operated animals sometimes had more fat cells surrounding the soleus muscle. It is unlikely that these produced any attenuation of the e.m.g., and they were easily distinguished from muscle cells in the light microscope. Small cells that appeared to be atrophied muscle fibres were also present in the operated muscles on the side from which muscle had been earlier removed. These were presumably fibres that became permanently denervated as a result of resection of intramuscular nerve branches at the time of operation, or some may have been portions of incompletely amputated fibres lacking innervation. Such atrophied fibres were not included when the number of muscle fibres was counted.

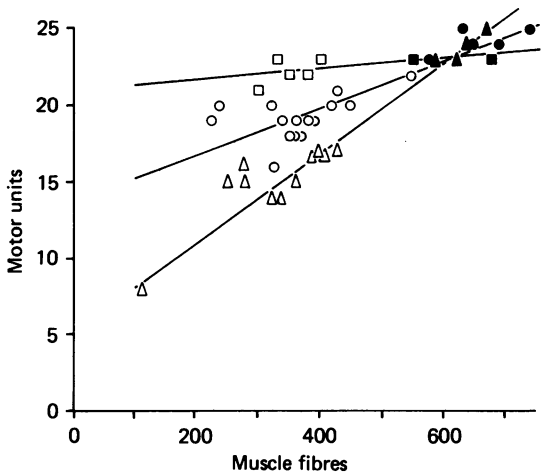


Fig. 1. Number of motor units plotted against number of muscle fibres for individual soleus muscles following operations at age 1–4 days (△—▲), 8–22 days (○—●) and 42–44 days (□—■). Open symbols: soleus nerve crushed and muscle surgically reduced in size. Filled symbols: soleus nerve crushed only. Least-squares regression lines are shown for the three sets of data. Correlation coefficients for muscles operated on at age 1–4 days ($r = 0{\cdot}97$) and at age 8–13 days ($r = 0{\cdot}82$) are highly significant ($P < 0{\cdot}001$) but at 42–44 days the correlation is not significant ($r = 0{\cdot}58$; $P > 0{\cdot}05$).

RESULTS

Motor units and muscle fibres in surgically reduced muscles

The numbers of muscle fibres and motor units surviving in muscles that were reduced in size at various post-natal ages are shown in Fig. 1. For convenience the data have been grouped according to the age at which the operations were performed: 1–4 days, 8–22 days and 42–44 days. Operation in the first few days of life results in a highly significant correlation between motor unit and muscle fibre numbers. The loss of motor units following operation in older animals (8–22 days) is also highly significant, but is less marked, while in the animals operated on at age 42–44 days there is no significant reduction in motor unit numbers following reduction in muscle size. Thus, in younger animals, the dependence of the motor neurones on muscle fibres is greater. However, even for the new-born animals this dependence is not strictly proportional, since the least-squares line does not pass through the origin (y-intercept is 6·2 units with 95% confidence limits of 4·3 and 7·8 motor units).

In Fig. 2 the change in dependence with age has been expressed in a manner that

does not require grouping of data. At any given age the loss of muscle fibres produces a certain loss of motor units, so a *dependence factor* can be defined as follows:

$$\text{dependence factor} = \frac{\text{fractional loss of motor units}}{\text{fractional loss of muscle fibres}},$$

$$\text{where fractional loss} = \frac{\text{mean control value} - \text{experimental value}}{\text{mean control value}}.$$

(The mean control value is the number of muscle fibres or motor units for all muscles where the nerves were crushed but the muscles were not reduced in size.)

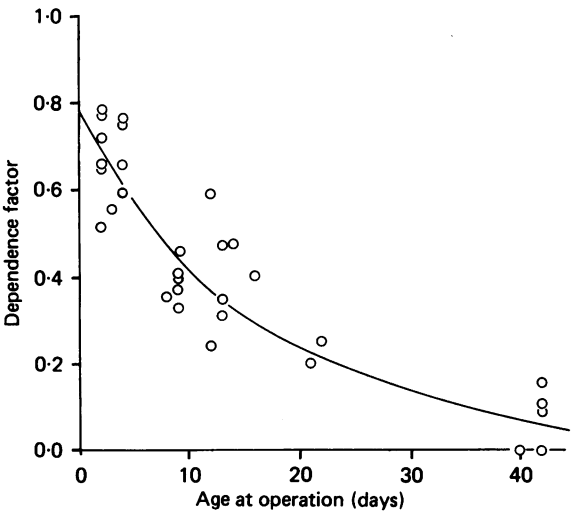


Fig. 2. Target dependence (for definition see text) of soleus motor neurones at different post-natal ages. Each point represents the target dependence derived from a soleus muscle surgically reduced in size at the particular post-natal age. Curve drawn by eye.

The dependence factor has a value of 0 when the loss of muscle fibres has no effect on motor units, and a value of 1·0 when a loss of muscle fibres is accompanied by a loss of motor units of equal proportion. Fig. 2 shows that the dependence factor approaches 0 by 40 days of age. The dependence at birth is approximately 0·8, and if the curve is extrapolated linearly there would be a dependence factor of 1·0 at about 4 days before birth.

Multiple innervation of muscle fibres

The method of counting step changes in the e.m.g. gives an estimate of the number of functional motor units in each muscle, provided that individual motor units are not obscured by extensive multiple innervation of muscle fibres. Therefore, where there were apparent reductions in the number of motor units it was important to determine that multiple innervation was at a low level. (In a muscle of twenty-five motor units a 4% level of multiple innervation could obscure one motor unit only if most of the fibres of that unit were multiply innervated. In practice much higher levels of multiple innervation would be required to obscure a motor unit.)

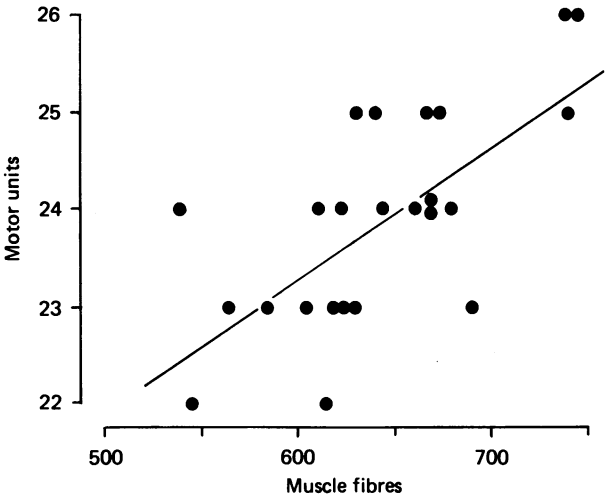


Fig. 3. The number of motor units and muscle fibres in individual normal soleus muscles. The correlation is significant ($r = 0{\cdot}70$; $P < 0{\cdot}001$) and the least-squares regression line is shown.

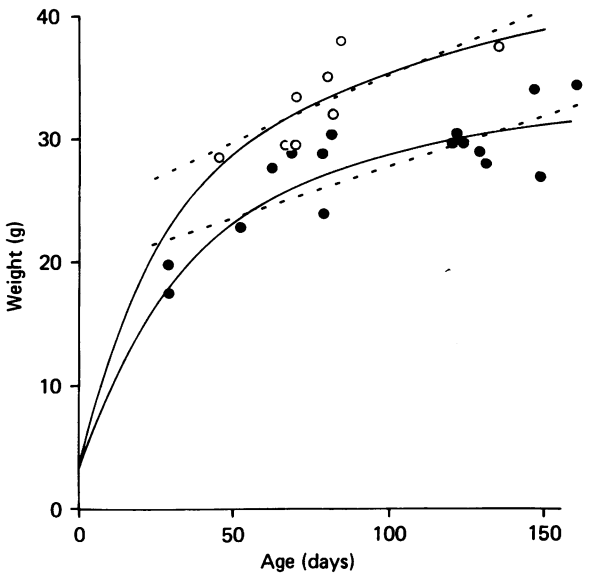


Fig. 4. Weight of female (●) and male (○) mice at different ages. There are significant linear correlations for female ($r = 0{\cdot}75$; $P < 0{\cdot}01$) and male ($r = 0{\cdot}75$; $P < 0{\cdot}05$) animals. The dashed lines are least-squares regression lines; the curves were fitted by eye. A datum point from a female aged 339 days was not included in this analysis.

The greatest reduction in the number of motor units occurred following reductions in muscle size at early post-natal ages (1–4 days). In all of these muscles, none of the thirty to forty muscle fibres had multiple end-plate potentials, and in four muscles stained for cholinesterase there were no multiple cholinesterase patches on individual muscle fibres. In the twenty muscles reduced in size at later ages, the maximum

proportion of multiple innervation assessed by either method was 10%. Thus, multiple innervation cannot be the cause of the reduction in motor units following operations in the young animals, and in the older animals it is unlikely that even a single motor unit would be obscured.

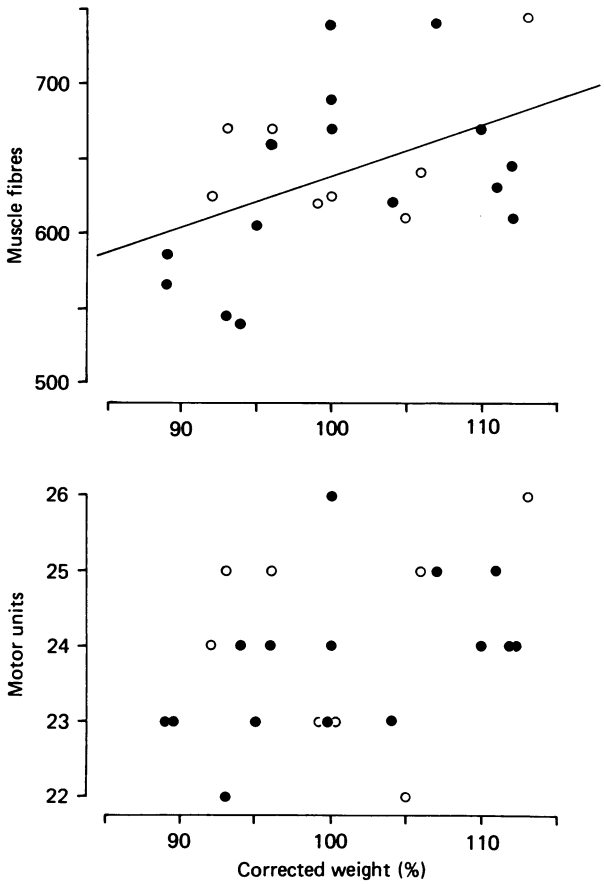


Fig. 5. Number of muscle fibres and motor units in soleus muscles of female (●) and male (○) mice plotted against weight of animal corrected for age (see text). The only significant correlation was for the combined female and male muscle fibre data (least-squares line shown; $r = 0{\cdot}46$; $P < 0{\cdot}05$).

Motor units and muscle fibres in normal muscles

Fig. 3 shows data for twenty-four normal muscles, which varied in size from 540 to 695 fibres. The number of motor units innervating these muscles ranged from 22 to 26, and the correlation between the numbers of fibres and units is highly significant. Once again the relation between units and fibres is not strictly proportional (the y-intercept of the least-squares line in Fig. 3 is 15 units with 95% confidence limits of 11 and 19 units).

The data for the normal animals were further analysed to determine whether the relation between numbers of fibres and units could be ascribed to the size of animals

(for example, larger than average animals might be expected to have more muscle fibres and motor units than average). Animal weight at time of sacrifice would be one index of animal size; however, animals were sacrificed over a range of ages during which weight was increasing (see Fig. 4), so it was necessary to derive a corrected weight for each animal and then to determine whether there were correlations between corrected weight and numbers of muscle fibres or motor units. The corrected weights were the actual weights at any given age expressed as a percentage of the weight predicted for that age from the curves in Fig. 4. The numbers of muscle fibres and motor units were then plotted against the corrected weights (Fig. 5). The correlation between number of fibres and corrected weight was poor, and there was no statistically significant correlation between units and corrected weight.

DISCUSSION

Before conclusions can be drawn about the survival of soleus motor neurones in the developing animal, it is necessary to consider whether the assay for motor units gives a reliable measure of the number of surviving motor neurones innervating the soleus.

Possible over-estimates of motor unit numbers resulting from alternation of motor unit recruitment were minimized as described in the Methods. Axon branching in the nerve proximal to the point of nerve stimulation would also yield an over-estimate of the motor units, because the branches would be recruited separately when the nerve was crushed. Underestimation would occur if some motor units were too small to be detected in the e.m.g. In the normal muscles the mean number of motor units was 23·9. This compares well with estimates of 21 units (Brown, Holland & Ironton, 1980) and 28 units (Caccia, Harris & Johnson, 1979), which were made using somewhat different methods, so there is probably no significant systematic error in our method of counting units in normal muscles.

In the experimental muscles the number of motor units would be over-estimated if branching was induced in the motor axons above the point of nerve stimulation. This is unlikely to have occurred here, since the point of nerve stimulation was in the sciatic nerve at least 15 mm proximal to the site of soleus nerve crush, and branching of myelinated axons is probably induced within only a few millimetres of the site of a peripheral nerve lesion (Aitken, Sharman & Young, 1947; Morris, Hudson & Weddell, 1972). Underestimates would occur if motor nerves regenerated into the muscles but failed to form motor units sufficiently large to be detected in the e.m.g. This does not happen in muscles reduced in size at age 42–44 days, since there is no significant reduction in motor unit numbers in these muscles, and there seems no reason *a priori* why motor axons surviving following muscle reductions in the younger animals should fail to form motor units large enough to be detected. Indeed, in neonatal animals failure to reconnect promptly with a target following nerve injury leads to the rapid degeneration of neurones (see review by Lieberman, 1974), so it is likely that neurones that reconnect with only a small number of fibres (too few to constitute a detectable motor unit) will not survive.

Assuming then, that the number of motor units in each muscle is equivalent to the number of surviving motor neurones, we have demonstrated that reduction in

the size of a muscle in neonates leads to the loss of motor neurones. There are a number of mechanisms by which this could occur.

1. The neurones that die have restricted physical access to the remaining muscle fibres compared with the neurones that survive. If motor units in the neonatal mouse soleus muscle are restricted to different regions of the muscle, then excision of part of the muscle might remove the entire peripheral field of some motor neurones and leave that of others intact. The neurones lacking a target might then be much more likely to die than those with a target, since as pointed out above, total removal of the target in neonates causes the death of the neurones. The distribution of motor units in the neonatal mouse soleus is not known but in cross-sections of the tibialis anterior muscle of the adult rat the fibres of individual units are scattered on average across an area of 4–5 mm^2 (Edstrom & Kugelberg, 1968). There are 128 muscle fibres per unit in this rat muscle compared with 27 in the mouse soleus, so if motor unit cross-sectional area is proportional to motor unit size, the area of a soleus motor unit would be 1 mm^2. This is considerably greater than the cross-sectional area of the muscle (0·6 mm^2, estimated from Pl. 1*A*). Moreover, motor units in neonatal muscles are distributed more widely than in the adult (Brown & Booth, 1983), so it is likely that the fibres of neonatal motor units in mouse soleus are not localized to different regions of the muscle. Motor units are unlikely to become regionalized when axons regenerate following the operations, since the mosaic pattern of distribution of motor units is re-established following nerve crush injury, at least in the adult (Karpati & Engel, 1968). This is consistent with the fact that axons are guided back to the sites of their target cells by their original Schwann cell pathways following a nerve crush (Weiss & Taylor, 1944). Furthermore, motor neurones regenerating following crush injury develop extra functional branches both in the adult (McArdle, 1975) and in the neonate (Brown *et al.* 1976). Taken together, these considerations make it likely that the motor neurones have equal access to the remaining muscle fibres following muscle reductions.

2. All the neurones have equal physical access to the remaining muscle fibres, but the neurones that die might be mismatched to these fibres. To explain his observations on cell death of the neurones innervating tadpole limbs, Lamb (1981) favours a model in which neurones contacting muscle cells of appropriate complementary identity survive, while neurones unable to contact complementary muscle fibres die. This mechanism is consistent with the present results, only if the muscle excisions result in the disproportionate loss of muscle fibres with particular identities. This seems unlikely, since fibres with particular identities would belong to particular motor units, which as shown above are probably not localized within part of the muscle.

3. All the neurones have equal physical access to the remaining muscle fibres, but the neurones that die are the losers in a competition between the neurones for the fibres. This requires that muscle fibres provide trophic support for a restricted number of motor neurones. A reduction in the number of muscle fibres in the experimental muscles would therefore result in the death of some motor neurones. This mechanism offers the most parsimonious explanation for the present observations.

In all of these possible mechanisms it has been assumed that in the neonate contact with a minimum number of muscle fibres is required to ensure the survival of motor neurones. Reductions in muscle size produce the greatest loss of motor units at birth,

and Fig. 2 predicts that a 1:1 relation between motor unit survival and muscle size occurs in the week preceding birth. This would coincide with the major period of motor neurone death in the lumbar spinal cord of the mouse (Lance-Jones, 1982), and so provides additional support for the competition mechanism. The apparent decrease in the dependence on muscle fibres in older animals could have several explanations. For example, muscle fibres in older animals might produce a greater quantity of some agent that is required for neurone survival, neurones may become intrinsically less dependent on such an agent, or the agent may be obtained from some other site. Any requirement for matching of identities of neurone and target cells could also be relaxed in older animals.

In normal muscles the correlation between the numbers of motor units and muscle fibres must reflect some kind of embryonic interaction, since there are no age-related changes in either of these variables after birth (Rowe & Goldspink, 1969; Brown *et al.* 1976). The correlation implies either that both variables are related independently to some third common cause, or that there is a direct causal relation between them. The only obvious independent variable that might be causally related to muscle and motor neurone cell numbers is animal size. If it is assumed that embryonic size differences are retained as adult weight differences, then a common causal connexion with embryonic size would imply the existence of correlations between weight and both muscle fibre and motor unit numbers. The poor correlations observed here between these variables make it unlikely that animal size is a common cause of the correlation between number of units and fibres.

A direct causal relation between the numbers of fibres and units would exist if the number of muscle fibres generated during development is dependent on the number of motor axons present in the muscle. The primary myotubes develop in the absence of innervation, but the development of the secondary myotubes requires the presence of functional motor axons within the muscle (Harris, 1981; McLennan, 1983). However, the full number of fibres in a rat distal limb muscle can develop post-natally with only 60% of the axons present (Betz, Caldwell & Ribchester, 1980) and in rat embryo diaphragm complete paralysis is required to prevent full development of the secondary myofibres (Harris, 1981). Thus, the motor nerves appear to play a permissive rather than instructive role in the proliferation of muscle cells, so it is unlikely that motor neurone numbers control the generation of the final number of muscle cells.

The only remaining explanation for the correlation is that the number of soleus neurones in each animal is determined in some way by the number of soleus muscle fibres. This could not happen at the time of generation of the motor neurones, since these are produced normally in the absence of the limb muscles (Lewis, Chevallier, Kieny & Wolpert, 1981). The motor neurone population would therefore have to be adjusted to the size of the muscle via the death of some neurones. This could occur by a competition between the motor neurones for occupancy of muscle fibres, or there could be a matching of neurone and muscle cell identities; in the latter case it would be necessary to postulate that variations in muscle size are accompanied by variations in the number of fibre identities present in the muscle.

In the experiments where the soleus muscle was reduced in size, the age-related change in the dependency of motor neurones on muscle fibres indicated that there

might be a strictly proportional relation between the sizes of neurone and muscle cell populations a few days before birth. Also, total removal of the muscles in neonates and embryos causes total loss of the motor neurones. However, the least-squares line for the normal muscle does not reveal a strictly proportional relation, since the line is considerably displaced from the origin. A similar non-linear relation has been observed between the numbers of granule cells and Purkinje cells in the cerebellum, where it is thought that the survival of the granule cells is controlled by the number of Purkinje cells present (Wetts & Herrup, 1983). The significance of this non-linear relation is not clear.

The New Zealand Medical Research Council provided financial support.

REFERENCES

AITKEN, J. T., SHARMAN, M. & YOUNG, J. Z. (1947). Maturation of peripheral nerve fibres with various peripheral connections. *Journal of Anatomy* **81**, 1–22.

BENNETT, M. R., MCGRATH, P. A., DAVEY, D. F. & HUTCHINSON, I. (1983). Death of motorneurones during the postnatal loss of polyneuronal innervation of rat muscles. *Journal of Comparative Neurology* **218**, 351–363.

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). Postnatal development of the adult pattern of motor axon distribution in rat muscle. *Nature* **304**, 741–742.

BROWN, M. C., HOLLAND, R. L. & IRONTON, R. (1980). Nodal and terminal sprouting from motor nerves in fast and slow muscles of the mouse. *Journal of Physiology* **306**, 493–510.

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.

CACCIA, M. R., HARRIS, J. B. & JOHNSON, M. A. (1979). Morphology and physiology of skeletal muscle in aging rodents. *Muscle and Nerve* **2**, 202–212.

COWAN, W. M. (1973). Neuronal death as a regulative mechanism in the control of cell number in the nervous system. In *Development and Aging in the Nervous System*, ed. ROCKSTEIN, M., pp. 19–41. New York and London: Academic Press.

EDSTROM, L. & KUGELBERG, E. (1968). Histochemical composition, distribution of fibres and fatiguability of single motor units. *Journal of Neurology, Neurosurgery and Psychiatry* **31**, 424–433.

HALL, E. K. & SCHNEIDERHAN, M. A. (1945). Spinal ganglion hypoplasia after limb amputation in fetal rat. *Journal of Comparative Neurology* **82**, 19–34.

HAMBURGER, V. (1958). Regression versus peripheral control of differentiation in motor hypoplasia. *American Journal of Anatomy* **102**, 365–410.

HAMBURGER, V. (1975). Cell death in the development of the lateral motor column of the chick embryo. *Journal of Comparative Neurology* **160**, 535–546.

HAMBURGER, V. & LEVI-MONTALCINI, R. (1949). Proliferation, differentiation, and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. *Journal of Experimental Zoology* **111**, 457–501.

HAMBURGER, V. & OPPENHEIM, R. W. (1982). Naturally occurring neuronal death in vertebrates. *Neuroscience Commentaries* **1**, 39–55.

HARRIS, A. J. (1981). Embryonic growth and innervation of rat skeletal muscles. I. Neural regulation of muscle fibre numbers. *Philosophical Transactions of the Royal Society, Series B* **293**, 257–277.

HARRIS, J. B. & WILSON, P. (1971). Mechanical properties of dystrophic mouse muscle. *Journal of Neurology, Neurosurgery and Psychiatry* **34**, 512–520.

HOLLYDAY, M. & HAMBURGER, V. (1976). Reduction of the naturally occurring motor neuron loss by enlargement of the periphery. *Journal of Comparative Neurology* **170**, 311–320.

KARNOVSKY, M. J. & ROOTS, L. (1964). A 'direct-colouring' thiocholine method for cholinesterases. *Journal of Histochemistry and Cytochemistry* **12**, 219–221.

KARPATI, G. & ENGEL, W. K. (1968). 'Type grouping' in skeletal muscles after experimental reinnervation. *Neurology (Minneapolis)* **18**, 447–455.

LAING, N. G. (1982). Motor projection patterns to the hind limb of normal and paralysed chick embryos. *Journal of Embryology and Experimental Morphology* **72**, 269–286.

LAMB, A. H. (1980). Motoneurone counts in Xenopus frogs reared with one bilaterally innervated hindlimb. *Nature* **284**, 347–350.

LAMB, A. H. (1981). Target dependency of developing motoneurones in *Xenopus laevis*. *Journal of Comparative Neurology* **203**, 157–171.

LANCE-JONES, C. (1982). Motoneuron cell death in the developing lumbar spinal cord of the mouse. *Developmental Brain Research* **4**, 473–479.

LANDMESSER, L. T. (1980). The generation of neuromuscular specificity. *Annual Review of Neuroscience* **3**, 279–302.

LEWIS, J., CHEVALLIER, A., KIENY, M. & WOLPERT, L. (1981). Muscle nerves do not develop in chick wings devoid of muscle. *Journal of Embryology and Experimental Morphology* **64**, 211–322.

LIEBERMAN, A. R. (1974). Some factors affecting retrograde neuronal responses to axonal lesions. In *Essays on the Nervous System*, ed. BELLAIRS, R. & GRAY, E. G., pp. 71–105. Oxford: Clarendon Press.

MCARDLE, J. S. (1975). Complex end-plate potentials at the regenerating neuromuscular junction of the rat. *Experimental Neurology* **49**, 629–638.

MCLENNAN, I. S. (1983). Neural dependence and independence of myotube production in chicken hindlimb muscles. *Developmental Biology* **98**, 287–294.

MORRIS, J. H., HUDSON, A. R. & WEDDELL, G. (1972). A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **124**, 76–203.

PURVES, D. (1980). Neuronal competition. *Nature* **287**, 585–586.

ROMANES, G. J. (1946). Motor localisation and the effects of nerve injury on the ventral horn cells of the spinal cord. *Journal of Anatomy* **80**, 117–131.

ROOTMAN, D. S., TATTON, W. G. & HAY, M. (1981). Postnatal histogenic death of rat forelimb motoneurons. *Journal of Comparative Neurology* **199**, 17–27.

ROWE, R. W. D. & GOLDSPINK, G. (1969). Muscle fibre growth in five different muscles in both sexes of mice. *Journal of Anatomy* **104**, 519–530.

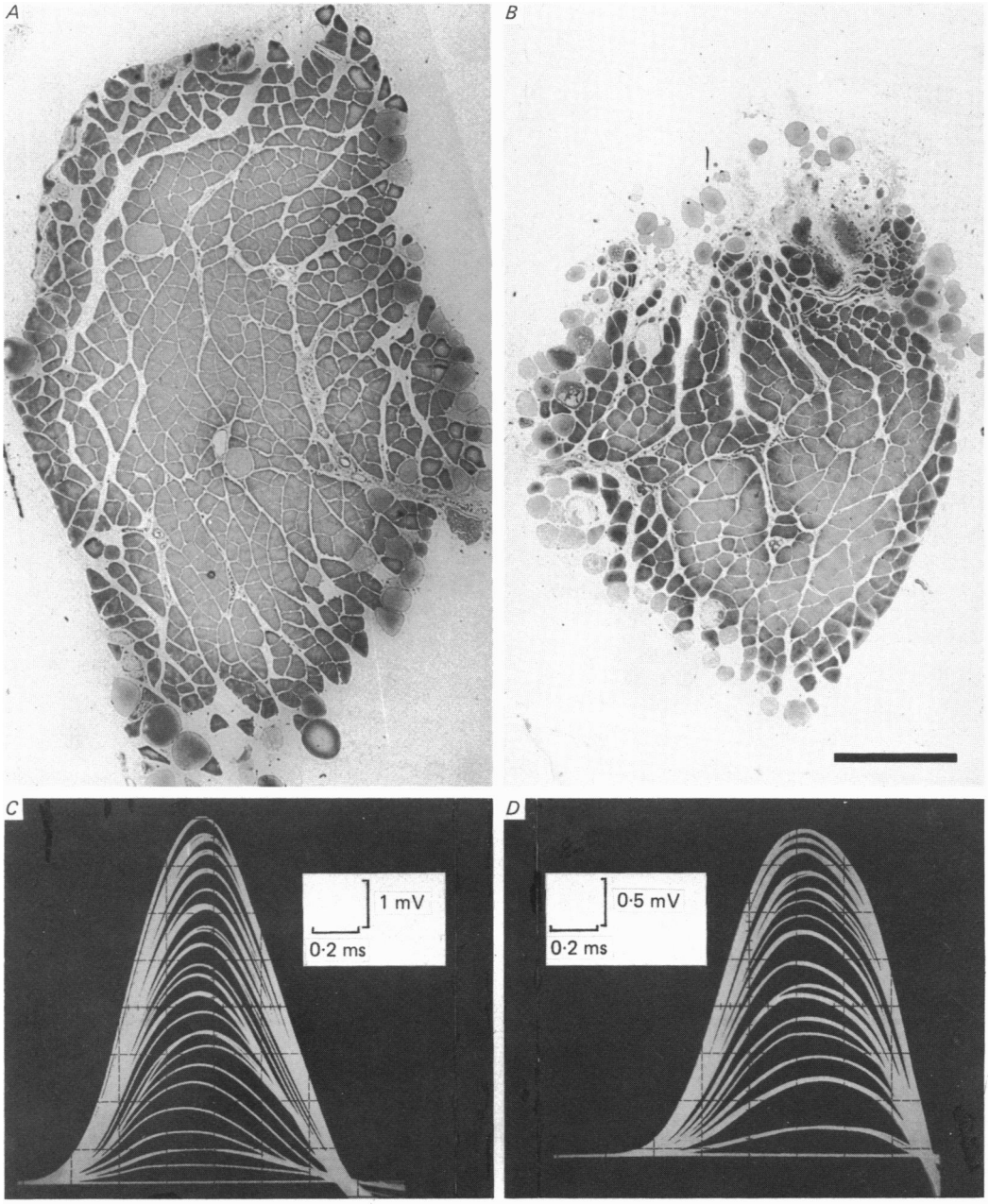
SLACK, J. R. & HOPKINS, W. G. (1982). Neuromuscular transmission at terminals of sprouted mammalian motor units. *Brain Research* **237**, 121–135.

WEISS, P. & TAYLOR, A. C. (1944). Further experimental evidence against neurotropism in nerve regeneration. *Journal of Experimental Zoology* **95**, 233–257.

WETTS, R. & HERRUP, K. (1983). Direct correlation between Purkinje and granule cell number in the cerebella of lurcher chimeras and wild-type mice. *Developmental Brain Research* **10**, 41–48.

EXPLANATION OF PLATE

A, cross-section of a normal soleus muscle at age 96 days showing 670 fibres. *B*, cross-section of a muscle at age 103 days reduced in size at age 12 days, showing 426 fibres. The lighter-staining cells around the edge of the muscle are fat cells. Bar, 200 μm. *C*, e.m.g. record from a normal soleus muscle recorded at age 96 days, showing 24 discrete units. *D*, e.m.g. record from a muscle reduced in size at age 4 days and recorded at age 100 days, showing 17 discrete units.



M. D. HABGOOD, W. G. HOPKINS AND J. R. SLACK

(*Facing p.* 314)