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# The effect of denervation and dystrophy on the adaptation of sarcomere number to the functional length of the muscle in young and adult mice

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## INTRODUCTION

During the postnatal growth of the mouse, increase in the length of certain limb muscles has been shown to result mainly from the serial addition of sarcomeres in the component muscle fibres (Williams & Goldspink, 1971). One likely stimulus for this increase in sarcomere number is the stretching of the muscle as the bones grow in length. Stretching of the muscle would be expected to stretch the sarcomeres: this would produce a decrease in the overlap of myosin and actin filaments, preventing the development of maximum contractile strength. There must be some kind of feedback mechanism enabling muscle fibres to adjust to a new length while retaining optimum sarcomere length by producing more sarcomeres in series.

However, in order for newborn muscle to adapt to increase in bone length, it would appear that movement of the muscle throughout its normal range is necessary, since, when newborn muscles are immobilized in an extended position, sarcomere addition is reduced. On the other hand, muscles from mice more than $2\frac{1}{2}$ weeks old, when immobilized in an extended position, are able to adapt to increase in bone length (Williams & Goldspink, 1973). It would seem that the excursion of the muscle is an important factor controlling sarcomere addition only in the first week or so of postnatal development.

It was decided to investigate further the factors governing growth in length of muscle fibres, in particular the role played by the nerve supply. Alder, Crawford & Edwards (1959) found that denervation of a limb muscle of a young rabbit resulted in a reduced muscle belly length, even though the muscle could still be moved passively. In the present study the effect of denervation on postnatal sarcomere addition in mouse muscle fibres has been studied. It was decided also to look at postnatal sarcomere addition in dystrophic mouse muscles. Whilst some authors consider that murine muscular dystrophy is primarily myogenic in origin (Parson, 1934; Cosmos, Butler & Milhorat, 1973; Hamburgh, Peterson, Bornstein & Kirk, 1975), there is evidence to suggest that dystrophic animals have an abnormality of the nervous system which may be largely responsible for the muscular disorder (McComas Sica, Upton & Petito, 1974; Gallup & Dubowitz, 1973; Peterson, 1974; Neerunjun & Dubowitz, 1975). It was therefore felt it would be interesting to determine how sarcomere addition in dystrophic muscles compares with that in both normal and denervated muscles.

By 8 weeks of age, sarcomere addition in the biceps brachii and soleus muscles of the normal mouse is virtually complete (Williams & Goldspink, 1971). However, muscle is a very adaptable tissue and sarcomere number can be altered (possibly in order to adjust sarcomere length) when the functional length of the muscle is changed by immobilization (Tabary *et al.* 1972; Williams & Goldspink, 1973). It has been shown that denervation does not prevent adult cat soleus muscles from adding on sarcomeres when the muscles are immobilized in an extended position. However, the soleus is a slow-twitch muscle made up mainly of type I fibres, and neurectomy in adult mammals has shown that type I fibres are less dependent on neuronal 'trophic' influence and do not undergo atrophy to the same extent, as type II fibres (Bajusz, 1964; Engel, Brooke & Nelson, 1966). It was decided to see whether the fast-twitch biceps brachii muscle is, when denervated, as capable of responding to change in functional length as the soleus muscle. It was also decided to determine whether adult dystrophic muscle can respond to an increased functional length, and to compare the extent and rate of any such adaptation with that of normal and denervated muscle.

## MATERIALS AND METHODS

All the mice used were of the 129/Re strain obtained originally from Jackson Memorial Laboratories, Bar Harbour, U.S.A. Both dystrophic homozygous and normal heterozygous mice were used. They were fed on Pilsbury's special breeding diet, with food and water available at all times. Dystrophic animals received a supplement of Bemax.

The soleus and biceps brachii muscles were chosen for this study because of their simple structure, with fibres which run from tendon to tendon. The soleus is particularly suitable for sarcomere number measurements since the variation in fibre length within a given muscle is very small. However, it was considered necessary also to study the biceps brachii in dystrophic mice since fast-twitch muscles such as the biceps brachii are affected by the disease to a greater extent than slow-twitch muscles such as the soleus (Rowe & Goldspink, 1969; Harris, 1971). A preliminary experiment (Table 1) showed that in the case of the soleus there is very little variation in sarcomere number, either within a muscle or between different muscles from animals of the same age (coefficient of variation = 0·24). In the biceps brachii there is greater variation in fibre length within a muscle, and so fibres were always selected from one particular region of the muscle. In this way the variation in the number of sarcomeres per fibre within a muscle, and between muscles from animals of the same age, was small (coefficient of variation = 0·28).

### *Determination of sarcomere number*

The method used to determine the number of sarcomeres along the length of single teased muscle fibres has been described in detail already (Williams & Goldspink, 1971). The mice were killed and the limbs were pinned so that the soleus and biceps muscles were in the lengthened position. The muscles were exposed and fixed *in situ* by 25 % glutaraldehyde. After fixation the muscles were placed in 30 % nitric acid to hydrolyse the connective tissue, then stored in 50 % glycerol. Individual whole

fibres were teased out and mounted in glycerine jelly. Using a Leitz projecting microscope the number of sarcomeres along the length of each fibre was counted.

*Postnatal sarcomere addition in denervated muscles*

3 day old animals underwent unilateral soleus denervation in consequence of the removal of a 2 mm length of the sciatic nerve. The animals were killed at intervals and the sarcomere number determined in both denervated and contralateral soleus muscles.

*Postnatal sarcomere addition in dystrophic muscles*

In the case of dystrophic muscle it was considered important to look at sarcomere number in relation to bone length, since if in dystrophic animals bone growth is reduced, a normal sarcomere number would not be expected. Female mice from 7 to 22 weeks old were used. Each animal was weighed, the sarcomere number determined for both biceps brachii and soleus muscles, and the length of the humerus and tibiofibula measured. Sarcomere counts were made only on those fibres which ran from tendon to tendon, i.e. severely atrophied fibres were excluded.

*The effect of denervation on sarcomere number in adult biceps brachii and soleus muscle fibres*

12 week old female mice were used. In one group of animals the sciatic nerve on one side was exposed and a 2 mm length removed. In a second group the nerve to the biceps brachii was exposed on one side and a portion removed. The animals were killed after 2 weeks and sarcomere number in the biceps brachii and soleus muscles was determined for the denervated and contralateral sides.

*The response of denervated adult muscles to immobilization in extension*

In one group of adult female mice the soleus of one side was denervated as described above. Using 'Gypsona' plaster of Paris bandage a plaster cast was put on each denervated hind limb so that the soleus was immobilized in a lengthened position. In a second group of animals the biceps brachii of one side was denervated and a plaster cast put on the denervated forelimb so that the biceps was immobilized in a lengthened position. The animals were killed at weekly intervals and sarcomere number in the biceps and soleus muscles was determined for the experimental and contralateral sides. In addition, some mice had their casts removed and the ability of the denervated muscles to recover from immobilization was determined.

*The response of adult dystrophic muscles to immobilization in extension*

Plaster casts were applied to 6 month old female dystrophic mice. The right forelimb and left hind limb of each animal were immobilized so that the soleus and biceps brachii muscles were held in lengthened positions. Between 1 and 2 weeks after immobilization mice were killed and the number of sarcomeres was determined. Two mice had their casts removed after a 2 week period of immobilization, and the ability of the muscles to recover was determined. The results of this experiment indicated that whilst dystrophic muscle is capable of adding on sarcomeres when immobilized in the lengthened position, it does so at a slower rate. Therefore, an

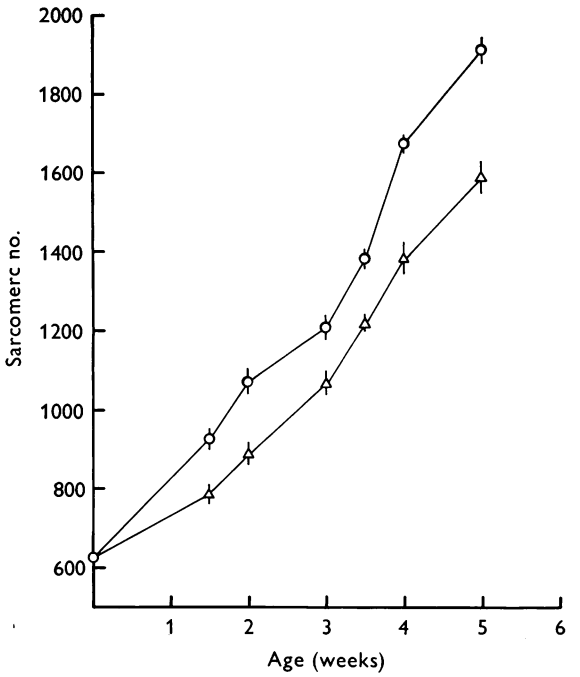


Fig. 1. Postnatal sarcomere addition in denervated muscle fibres. The animals underwent unilateral section of the sciatic nerve when they were 3 days old. △, denervated and ○, contralateral soleus muscle fibres. Each point is the mean of three readings ∓ standard error of the mean.

additional experiment was carried out to determine more exactly the rate and extent of sarcomere addition. The soleus muscles from one side of dystrophic and normal male mice were immobilized in the lengthened position. The animals were killed at frequent intervals and the sarcomere number determined in immobilized and contralateral muscles. Counts were made on five fibres from each muscle.

## RESULTS

### *Postnatal sarcomere addition in denervated muscles*

Denervated muscles contained much connective tissue and the diameter of the muscle fibres was greatly reduced. However, even five weeks after denervation the majority of fibres ran from tendon to tendon and contained clearly visible sarcomeres The postnatal addition of sarcomeres in the denervated muscle fibres was found to fall short of that in the contralateral muscles (Fig. 1). However, the reduction was not as great as that caused by immobilization (see Williams & Goldspink, 1973).

### *Postnatal sarcomere addition in dystrophic muscle fibres*

The body weights of the dystrophic mice had values which were much lower than those of normal animals, and at 22 weeks the dystrophic mice weighed only half as much as the controls (Fig. 2*c*). In spite of this the lengths of the limb bones were not

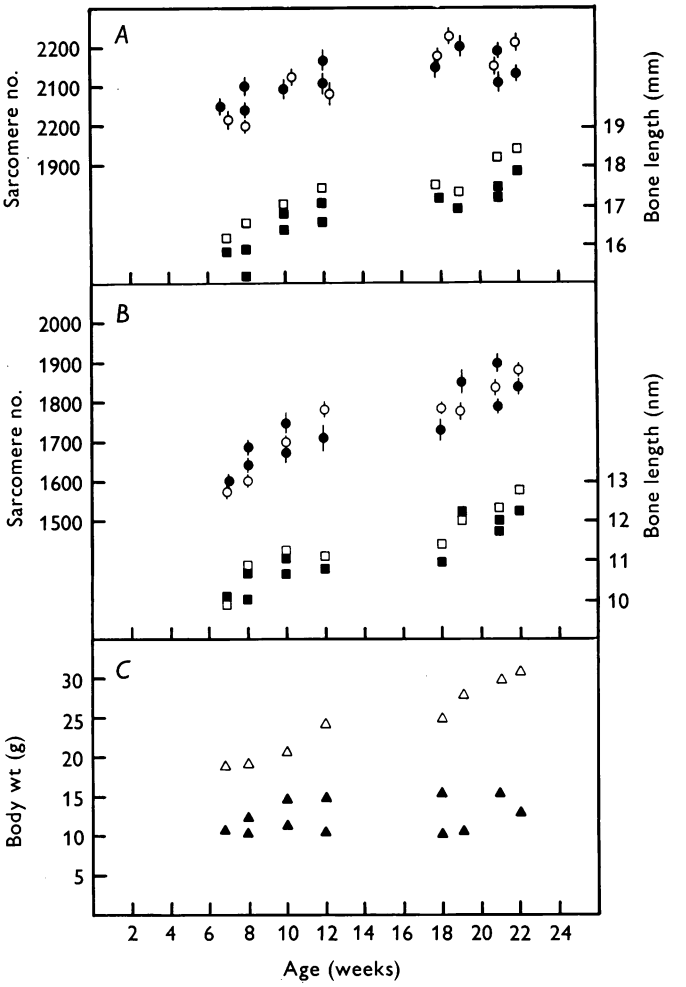


Fig. 2A, Sarcomere number in soleus muscle fibres and the length of the tibio-fibula from dystrophic and control mice. ●, dystrophic, and ○, control sarcomere number. ■, dystrophic, and □, control bone length. B, sarcomere number in biceps brachii muscle fibres and the length of the humerus from dystrophic and control mice. ●, dystrophic, and ○, control sarcomere number.■, dystrophic and □, control bone length.
C, Body weight in dystrophic (▲)and control (△)mice. Each point represents the data from one animal.

very different from those of the controls. Similarly, the number of sarcomeres per fibre in both biceps and soleus muscles did not differ significantly from normal (Fig. 2*A*, *B*).

Whilst teasing the dystrophic muscles several features were noted which were not seen in the normal muscles. Although the majority of fibres ran from tendon to tendon, some fibres were short, of very small diameter, and in parts had sarcomeres which were scarcely visible. (These fibres were excluded from the experiment.) There was a big variation in the girth of the fibres, even in the soleus muscle which, in

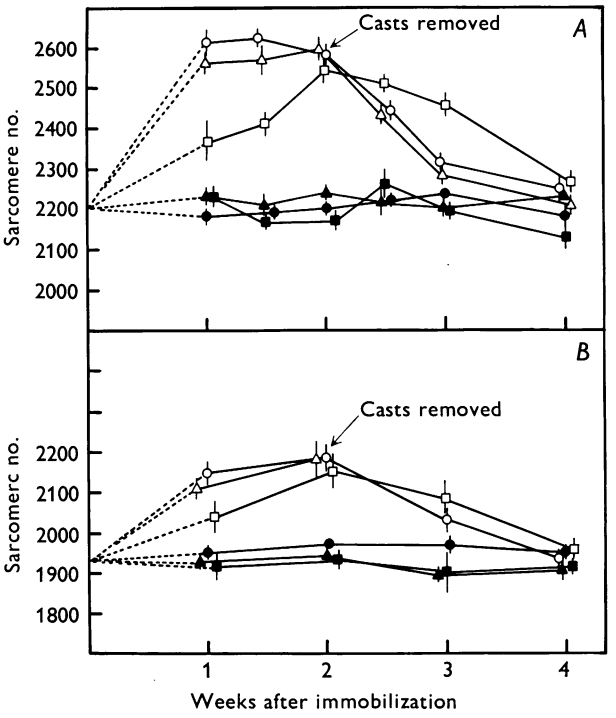


Fig. 3. Sarcomere number in adult muscle fibres which had been immobilized in the lengthened position. The ability of the muscles to recover after removal of the casts (↑) was also followed. A, soleus muscle fibres; B, biceps muscle fibres. ○, normal immobilized, and ●, contralateral muscle fibres; □, dystrophic immobilized, and ■, contralateral muscle fibres; △, fibres from muscles which were denervated then immobilized, and ▲, contralateral muscle fibres which were denervated only. Each point is the mean of three readings from one muscle ∓ standard of the mean error.

normal animals, contains fibres of fairly uniform diameter (Rowe & Goldspink, 1969). Many of the fibres were seen to be split longitudinally (Fig. 4), sometimes over very long distances. (Fibre splitting in dystrophic muscle has also been described by Isaacs, Bradley & Henderson, 1973.) In animals which were extremely sick with dystrophy, however, no splitting fibres were observed.

*The effect of denervation on sarcomere number in adult biceps brachii and soleus muscle fibres*

Denervation had no effect on sarcomere number in either biceps brachii or soleus muscle fibres (Table 1).

*The response of denervated adult muscles to immobilization in extension*

Denervation did not affect the ability of biceps brachii and soleus muscles to respond to immobilization in extension by adding on more sarcomeres in series. As in normal muscle, the soleus muscle fibres added on more sarcomeres than the biceps brachii muscle fibres. After removal of the casts the sarcomere number returned to normal (Fig. 3).

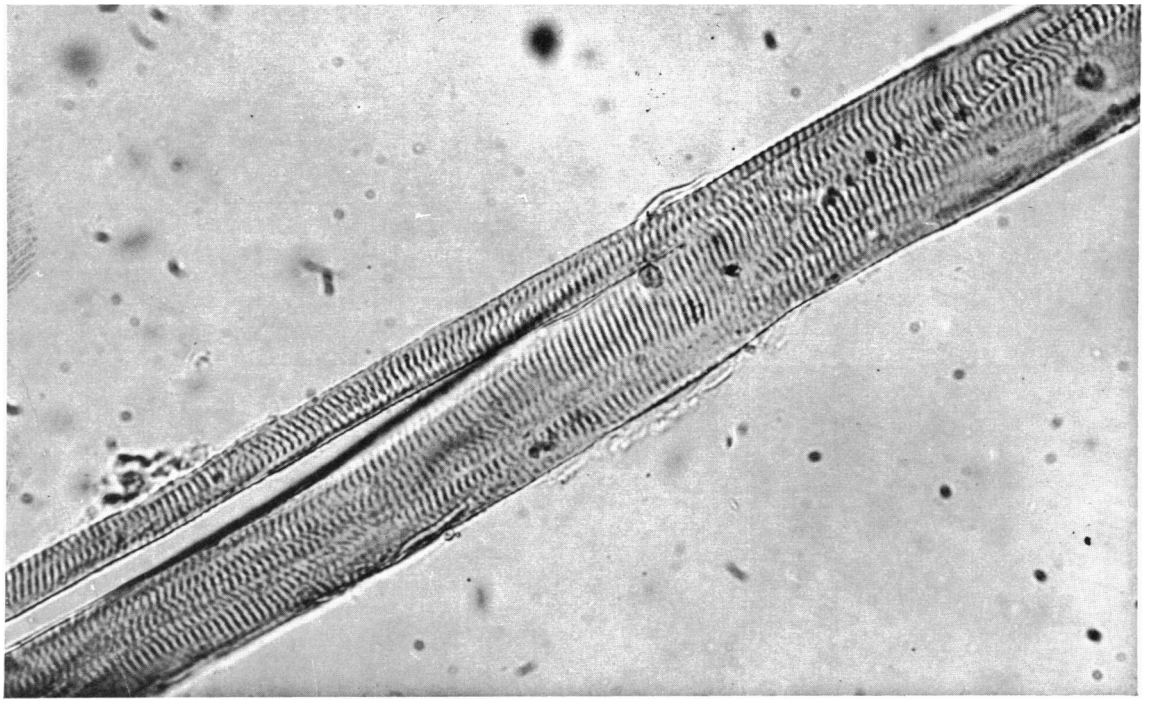

Fig. 4. A single teased fibre from a dystrophic soleus muscle showing longitudinal splitting. × 500.

Table 1. *The number of sarcomeres in soleus and biceps brachii muscle fibres from normal, dystrophic and denervated muscles*

(The right and left biceps brachii and soleus muscles of 3 adult normal and 3 adult dystrophic mice were used: 3 right or left biceps brachii and soleus muscles were taken from 3 adult mice whose muscles had been denervated. In each case measurements were made on 5 fibres from each muscle.)

| | Soleus | | | Biceps brachii | | |
|---|---|---|---|---|---|---|
| | Normal | Dystrophic | Denervated | Normal | Dystrophic | Denervated |
| No. of observations | 30 | 30 | 15 | 30 | 30 | 15 |
| Mean sarcomere number | 2245 | 2175 | 2237 | 1930 | 1873 | 1912 |
| Standard error of mean | 7·65 | 8·16 | 7·10 | 7·54 | 9·12 | 10·53 |
| Coefficient of variation | 1·87 | 2·06 | 1·74 | 2·14 | 2·67 | 2·79 |

*The response of adult dystrophic muscles to immobilization in extension*

Adult dystrophic muscle fibres were found to add on sarcomeres when immobilized in the extended position (Fig. 3 and Table 2). Although the number of sarcomeres produced was the same as in normal immobilized muscle, the results showed that the rate of production was lower. In muscles from normal animals there is a significant

Table 2. *Sarcomere number in soleus muscles from normal male and dystrophic male mice*

(In each case the soleus muscle of one limb was immobilized in the lengthened position. Each datum is the mean of 5 observations ∓ standard error of the mean. A one sample T test was used to compare the sarcomere number in immobilized and contralateral muscles. *indicates a significant difference between the pair of data ($P < 0.01$).)

| Days after immobilization | Mean sarcomere number | | | |
|---|---|---|---|---|
| | Dystrophic | | Normal | |
| | Immobilized (±S.E.) | Contralateral (±S.E.) | Immobilized (±S.E.) | Contralateral (±S.E.) |
| 2 | — | — | 2165 26 | 2243 14 |
| 3 | — | — | 2304 30 | 2272 26 |
| 4 | 2148 16 | 2105 27 | 2475 43* | 2265 18 |
| 5 | 2204 28 | 2247 36 | 2482 27* | 2223 12 |
| 5 | 2163 37 | 2184 41 | 2513 40* | 2304 16 |
| 7 | 2308 14* | 2223 18 | 2476 25* | 2242 23 |
| 8 | 2279 21 | 2239 23 | 2717 34* | 2292 18 |
| 10 | 2418 26* | 2150 17 | 2569 22* | 2250 11 |
| 12 | 2380 30* | 2164 18 | — | — |
| 13 | 2453 35* | 2236 24 | — | — |
| 14 | 2502 17* | 2266 10 | 2598 19* | 2207 13 |
| 17 | 2482 11* | 2186 11 | 2390 9* | 2197 11 |
| 21 | 2438 12* | 2204 17 | 2240 12 | 2165 14 |

increase in sarcomere number after four days of immobilization, whereas in dystrophic muscle it is not until after ten days of immobilization that sarcomere number is significantly increased.

## DISCUSSION

In muscle which is denervated at birth the fibres atrophy and this involves a decrease in fibre diameter and a loss of myofibrils (Muscatello, Margreth & Aloisi, 1965).

Similarly, in dystrophic muscle there is a loss of fibres and (in the biceps brachii) a reduction in the diameter of the fibres (Rowe & Goldspink, 1969). However, the experiments described here show that, in spite of being in a state of atrophy, longitudinal myofibril growth can occur in both denervated and dystrophic muscle fibres when the functional length of the muscle is increased either by postnatal bone growth or by immobilization in extension. Thus it would seem that the stimuli for transverse muscle fibre growth (hypertrophy) differ from those for longitudinal growth.

Denervation soon after birth, whilst not preventing the postnatal addition of sarcomeres, does result in a significant reduction in sarcomere number. (It is not certain whether it is the extent or the rate which is reduced: it was not considered wise to look at muscles from mice which were more than 5 weeks of age since it has been shown that considerable re-innervation occurs after this time; Engel & Karpati, 1968). The reduced sarcomere addition in denervated muscle might be due to the fact that an intact nerve supply is necessary for complete postnatal sarcomere addition. Alternatively, sectioning the sciatic nerve, and the resultant denervation of many of the hind limb muscles, may reduce the excursion of the muscle. Since it would

appear that movement of a muscle throughout its normal range is a factor of great importance for early postnatal sarcomere addition (Williams & Goldspink, 1973), reduced excursion may explain reduced sarcomere number. To distinguish between these two possibilities it would have been necessary to denervate by cutting only the nerve to the soleus; this would not have caused a significant alteration in the gait of the animal. Unfortunately, the small size of the newborn mouse makes this impracticable.

In young dystrophic muscle, postnatal sarcomere addition was normal. However, whilst teasing the muscle some short fibres were noted (but were excluded from the experiment). It is possible that these fibres were the ones most affected by the dystrophic process and were unable to add on the normal complement of sarcomeres. The many split fibres noted in the dystrophic muscle might be relatively normal fibres which are compensating for the atrophying short dystrophic fibres (splitting is generally considered to be a function of over-loading; Edgerton, 1970; Hall Craggs, 1970). However, the very small number of short fibres in comparison with the very large number of split fibres makes it seem unlikely that the former represent the total dystrophic fibre population. It is also interesting to note that, even though in very sick animals no split fibres are seen (this was also noted by West & Murphy, 1960), the fibres that are present are still able to produce the normal complement of sarcomeres.

The immobilization experiments described here show that normal adult muscle adapts to an increased functional length by adding on sarcomeres in series, and that both fast-twitch and slow-twitch muscles show this response. The fact that the soleus (slow-twitch) added on a greater percentage of sarcomeres than the biceps brachii (fast-twitch) can be explained by the fact that the former has the greater excursion, and in the *in vivo* lengthened position its sarcomeres are pulled out to a greater extent: thus more sarcomeres would have to be added on to give optimum sarcomere length.

Adult denervated muscle adjusts to increased functional length in the same way as normal muscle; when denervated and immobilized in the extended position both biceps brachii and soleus added on as many sarcomeres as muscles which had been immobilized only. This result confirms for fast-twitch muscle, the results of Goldspink *et al.* (1974) for the slow-twitch soleus muscle. Thus alterations in fibre length in adult muscle would seem to be independent of neural control.

Adult dystrophic muscle is also able to respond to increased functional length. When immobilized in the lengthened position, fibres from both the fast-twitch biceps brachii and the slow-twitch soleus added on sarcomeres. However, whilst the increase in sarcomere number was the same as in normal immobilized muscle, the rate at which the sarcomeres were added on was reduced. Similarly, the rate at which the muscles recovered from immobilization was reduced. The reasons for this are not known, but, since denervation does not affect a muscle's ability to respond to immobilization, it seems unlikely that the nervous system is directly involved.

The factors which control the remarkable ability of a muscle to add on sarcomeres even when it is affected by dystrophy, or deprived of neural input, still need to be examined at the biochemical level.

SUMMARY

In young animals the elongation of the limb bones increases the functional lengths of the muscles. In adult animals the functional length of a muscle can be increased by immobilizing it in the lengthened position. In both cases the muscle adapts by adding on more sarcomeres in series. The role of the nerve supply in this adaptation has been investigated using denervated muscles and muscles from dystrophic animals where there is thought to be an abnormality of the nerve supply.

Postnatal sarcomere addition in denervated muscles falls short of that of controls. Although this might mean that the nerve supply is necessary for normal addition of sarcomeres, it is just as likely that there is a change in gait resulting from denervation, which affects the sarcomere number. Sarcomere number in fully grown mice is not affected by denervation, nor is the ability of the muscle to adapt to immobilization in the lengthened position. This is true for fast-twitch as well as slow-twitch muscles.

In dystrophic muscles postnatal sarcomere addition is normal, although the presence of a few short fibres in the muscle may mean that some muscle fibres cannot adapt to an increase in the functional length of the muscle accompanying bone growth. Adult dystrophic muscle is capable of adapting to immobilization in the lengthened position. However, although the total number of additional sarcomeres is the same as in normal immobilized muscle, they are added on at a slower rate.

The experiments show that although denervated and dystrophic muscle fibres are in a state of atrophy they are still capable of adding on sarcomeres in series when the functional length of the muscle is increased. It would appear that the mechanism which enables the muscle to respond in this way to an increased functional length does not involve the nerve supply.

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