SARCOMERE LENGTH DURING POST-NATAL GROWTH OF MAMMALIAN MUSCLE FIBRES

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SUMMARY

The length of the sarcomeres, the A- and the I-filaments and their percentage overlap were measured in the fibres of the biceps brachii muscle from mice of different ages. The sarcomere length with the limb in the fully extended position was found to increase from 2.3 μ in the newborn animal to 2.8 μ in the adult. This increase was due to a decrease in the percentage overlap of the filaments and not to any change in the filament lengths. The sarcomeres at the ends of the fibres were found to be shorter than those in the middle of the muscle, at all ages. When the muscles were stretched beyond their resting length, only about the middle 60% of the sarcomeres in the young muscles increased in length. Length/tension plots were obtained for young and old muscles and the difference in the shape of these plots could be explained as being due to the non-functional terminal sarcomeres of the young muscles. The maximum tension developed by the young muscles was found to be attained at an initial muscle length about 10% greater than their length at maximum limb extension. The adult muscles developed maximum tension at their length at maximum limb extension.

INTRODUCTION

In most species of mammals the length of the limbs in the adult is about two or three times greater than in the newborn animal. The question arises as to how the muscle fibres and in particular how the myofibrils increase in length during the post-natal growth period. The structural composition of the myofibrils as an interdigitating hexagonal array of thick and thin filaments organized in repeated units called sarcomeres (Hanson & Huxley, 1953) is now very well established. The gross mechanical events accompanying the shortening of the myofibrils which involve the sliding of the thin filaments over the thick filaments (A. F. Huxley & Neidergerke, 1954; H. E. Huxley & Hanson, 1954; Hanson & Huxley, 1955) also seem beyond dispute. In view of this available knowledge it was decided that not only would the structural changes accompanying the lengthening process of the myofibrils be studied but that the physiological significance of these changes would also be investigated.

The author (Goldspink, 1964) had previously carried out an investigation into the mechanism of the longitudinal growth of mouse muscle fibres using light microscopy. It was found that the increase in length of the fibres was due to an increase in both the number and the length of sarcomeres until the mouse was about 3 weeks old (12 g body weight). Thereafter the increase in the length of the muscle was mainly if not entirely due to an increase in the length of the individual sarcomeres. This previous study was however incomplete as it was only possible with the light micro-
scope to measure the change in the mean sarcomere length. The work described below is an electron-microscope study of this post-natal change in sarcomere length and its physiological implications.

MATERIALS AND METHODS

The mice used in this investigation were all males of the TT strain originally obtained from Tuck and Sons, England. They were maintained on modified diet formula 41b (Oxoid Ltd), with food and water available at all times. The biceps brachii was the muscle chosen for this study as it has a relatively simple structure with fibres running from tendon to tendon and by observing the limits of the tendons it was possible to estimate the length of the fibres. Another consideration was that this muscle in the mouse was small enough to be fixed whole and in situ.

Electron microscopy. Muscles used for electron-microscopic examination were prepared in one of two ways. In one series, mice of different ages were killed by dislocation of the cervical vertebrae and pinned out on a cork board with their forelimbs in the fully extended position. Care was taken, particularly in pinning out the limbs of very young mice, so as not to stretch the muscles beyond their maximum in vivo resting length by causing dislocation of the elbow joint. They were then placed in a cold room at 4 °C and allowed to go into rigor in this position. The purpose of maintaining the carcasses at 4 °C was to avoid violent post-mortem changes, especially local contractions of the fibres (Bendall, 1960) and also to minimize bacterial action. After 24 h the carcasses were taken out of the cold room and the skin and connective tissue were removed from the forelimbs to expose the biceps brachii muscle. The forelimbs were then severed from the carcase and pinned to a smaller piece of cork (still in the fully extended position) and immersed in fixative.

Another series of mice ranging from newborn animals to adults was sacrificed and the biceps brachii muscles were fixed as soon as possible after death. In this case the forelimbs were quickly skinned, removed, pinned to a piece of cork in their fully extended position and immersed in fixative.

The fixative used in both cases was a 2·5 % glutaraldehyde solution made by diluting a 25 % purified, commercial solution with Ringer Locke and buffered to a pH of 7·3 with 0·1 M phosphate buffer. After 1½ h the muscles were dissected from the bone and replaced in the fixative for a further 1½ h. They were then washed overnight in 0·1 M phosphate buffer solution and post-fixed for 2 h in Palade's OsO₄ fixative. The muscle fibres were then teased apart in 70 % ethanol. After dehydration in 100 % ethanol, short lengths of single muscle fibres or small bundles of fibres from the middle of the muscle were embedded in Araldite. Sections of approximately 500 Å thickness were cut on a diamond knife using a Huxley Cambridge Ultra-microtome with the knife edge parallel to the fibre axis. These were collected on carbon-coated grids, stained in potassium permanganate and lead citrate solutions (H. E. Huxley, 1965, personal communication) and examined with a Siemens Elmiskop 1 A electron microscope. Thinner sections showing silver interference colours were also occasionally examined but these proved to be less convenient for routine measurement purposes. Measurements of sarcomere length, A- and I-filament lengths and
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the percentage overlap of the A-filaments by the I-filaments were made from electron micrographs using micrometer calipers.

**Light microscopy.** Muscles for examination with the light microscope were fixed *in situ* immediately after the animals were killed, using the same procedure as for the electron microscopy. To investigate the effect of stretching a muscle beyond its resting length the forelimb was pulled gently until the elbow joint just became dislocated. It was then pinned out and fixed in this position.

After dehydration the muscles for light microscopy were embedded in ester wax (Steedman, 1960) and serial longitudinal sections were cut at a thickness of 4 μ. The sections were stained in Heidenhain’s iron haematoxylin and mounted in Canada Balsam. Sarcomere length measurements were carried out on sections through the middle of the longitudinal axis of the muscles by counting the number of A-bands along a 80 μ length of fibre. The 80-μ length was measured using an ocular micrometer scale aligned to coincide with a Z disc. Counts were carried out usually to the nearest third of a sarcomere. The position along the muscle at which the measurement was made was read from the millimetre vernier scale on the stage of the Leitz Ortholux microscope.

**Length/tension measurements.** The length/tension characteristics of very young and adult biceps brachii muscles were measured *in vivo* using photo-electric myographs and pen recorder (E and M Instruments, Texas). The animals were first anaesthetized with nembutal using the dosage recommended by Pilgrim & De Ome (1955). The forelimbs were firmly affixed to a cork board and the overlying skin was removed to expose the biceps brachii. The muscle length at maximum limb extension was then measured with micrometer calipers. The origin tendon of this muscle was then cut and connected to the myograph with a short length of silk thread. As the nerve supply and the blood supply enter the muscle about mid-way down the inner side, the muscle was only slightly raised to attach it to the myograph. In other words the muscle was at an angle of about 20° to the forelimb and hence the blood supply and nerve supply remained intact throughout the period of the measurement procedure. Two fine silver-wire electrodes were placed under the muscle so that they were in contact with the nerve. The muscle was then stimulated via the nerve to contract tetanically for brief periods by delivering short bursts of pulses (5 V d.c. square wave of 1 msec duration) at a frequency of 100/sec whilst it was held at different initial lengths. The initial length of the muscle was adjusted by the micrometer screw on the myograph. The change in the length of the muscle was read off from the scale on the micrometer. The myographs and pen recorder were calibrated by hanging a series of weights on to the attachment hook of the particular myograph. A type 'B' myograph with a spring displacement of 0·08 mm/g was used for the muscles of the very young mice and a type 'C' myograph with a spring displacement of 0·005 mm/g was used for the muscles of the mature mice. It was calculated that at the maximum tensions recorded myograph B allowed a 5% shortening of the muscles, and myograph C allowed a shortening of 1·5%. In each case the active tension was obtained by subtracting the tension due to the passive stretching of the muscle from the total recorded tension.
RESULTS

The results of the measurements of sarcomere length are shown in Figs. 1 and 5. They show that the length of the sarcomeres increases during post-natal growth from a value of 2.3 μ in the newborn mouse to about 2.8 μ in the mature mouse. It will also be seen from Figs. 1 and 5 that the reason for this change in length was that the extent of the overlap between the A- and the I-filaments had decreased. The

![Graph showing sarcomere length measurements](image)

Fig. 1. Sarcomere length measurements plotted against muscle fibre length: •, muscles fixed immediately after death; X, muscles fixed post rigor. The percentage overlap of the total length of the A-filaments by the I-filaments on each side is also given: •, muscle fixed immediately after death; x, muscles fixed post rigor. The plot at the top of the figure gives values for the total number of sarcomeres plotted against the fibre length. The values were obtained by dividing the fibre length by the sarcomere length for each muscle (○).

length of the A- and the I-filaments was found to be unchanged throughout the animal’s lifetime. The approximate number of sarcomeres along the length of the fibres at different stages of growth (Fig. 1) was calculated by dividing the fibre length by the sarcomere length. From Fig. 1, it will be seen that the most marked increase in the number of sarcomeres is during the period in which the muscle fibres grow from 4 to 6 mm in length.
The sarcomere length at different distances along the muscle was also investigated using the light-microscopy technique described above. Some of the results of this investigation are shown in Fig. 2. In this figure the sarcomere lengths at different points along the muscle are given for young and mature muscles fixed at maximum limb extension and also in a slightly stretched position. In addition to these a plot is given for a very old mouse (55 g mouse, approximately 18 months old). In all cases the sarcomeres at the ends of the fibre were shorter than those in the centre of the muscle. When the young muscles were stretched it was found that only a relatively small number of sarcomeres in the middle of the muscle were pulled out. In contrast to this situation, when the mature muscles were stretched all the sarcomeres increased in length, although the terminal sarcomeres were still shorter than those in the middle of the muscle. In the case of the very old muscles fixed at resting length the plot showed a very wide plateau with only a small percentage of shorter sarcomeres at the ends of the fibres.
In general the measurements of sarcomere length showed considerable variation, even those from the middle of the muscles. It was therefore felt desirable to plot frequency histograms of sarcomere length using measurements taken from light-microscopy sections. Histograms constructed for 100 measurements from the middle of five different muscles from young mice (2–6 g) and from mature mice (30–40 g) are given in Fig. 3. The sarcomere lengths in the young muscles showed a greater variation than in the mature muscles. However, in both cases most of the variation was attributable to variation between the different muscles and not between fibres of the same muscle. It will be noted that in the young muscles, as well as the greater scatter of the measurements, there was a tendency to exhibit a bimodal distribution.

The results of the measurements of the maximum contractile strength of the muscle at different initial lengths are represented by the plots in Fig. 4. Two of these plots are for young muscles from mice weighing approximately 3–6 g and the other two plots are for mature muscles from mice weighing approximately 40 g. It will be seen that the length/tension curves for the mature muscles show that the tension is less dependent on initial length for the range of plus or minus 20%. However for the young muscles the tension developed showed a marked dependence on the initial muscle length over the whole range. Also it will be noted that the
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Mature muscles developed maximum tension at their resting length (maximum limb extension) whereas the very young muscles did not develop maximum tension until they were pulled out a further 10–20% from their maximum in vivo length. Length tension plots for muscles slightly older than those presented for the young muscles showed that the maximum tension had moved to the maximum limb extension length by the time the animal had reached 7.0 g body weight (approximately 12 days old).

DISCUSSION

The conclusion from the work described here is that the length of the individual sarcomeres of the myofibrils in the mouse biceps brachii muscle increases from about 2.3 µ to 2.8 µ at maximum resting length during post-natal growth. Previously, an increase in sarcomere length during growth has only been reported (apart from Goldspink, 1964) as occurring in arthropod muscles (Aronson, 1961; Shafiq, 1963; Auber, 1965). The increase in sarcomere length in the mouse accounts for approximately 25% of the increase in muscle fibre length after birth; the other 75% is due to the formation of new sarcomeres along the length of the fibre. In the mouse the formation of new sarcomeres continues rapidly for several weeks after birth and this is in fact the period during which the most rapid increase in limb length takes place. During this period there was a 2-fold increase in muscle length (3–6 mm). After
6 mm the number of sarcomeres apparently continues to increase but at a very much slower rate: however, because of the considerable variation in the sarcomere length measurements it is not possible to say whether this calculated increase is a real increase or not. Also this apparent increase in the number of sarcomeres beyond the 6 mm fibre length stage may be due to the pulling out of some of the terminal sarcomeres (Fig. 2). Previous studies with the light microscope (Goldspink, 1964) suggested that the number of sarcomeres does not increase throughout the entire growth period of the muscle.

The formation of new sarcomeres in series will not be discussed here in detail as this investigation provided no direct evidence concerning its mechanism. However, it would seem that the most likely method is for the new sarcomeres to be formed at the ends of the growing myofibrils. Indeed, this may be the reason why the sarcomeres at the ends of the fibres are shorter than those in the middle. This may also be the reason why in the young muscles only the middle sarcomeres pull out when the muscle is stretched; in other words, the terminal ones are not as yet fully functional. Unfortunately this cannot be stated with any degree of certainty as it is not known to what extent the terminal sarcomeres are prevented from lengthening by the connective tissue which surrounds the fibres. Evidence presented by Fischman (1967) suggests that the aggregation of thick and thin filaments into the lattice of the sarcomeres is determined by the actin combining cross-bridges on the thick filaments. If this is the case then it may be expected that the first myofibrils would be laid down at a sarcomere length of 2.2–2.3 μ, at which there is a maximum interaction between the thick and the thin filaments. It will be seen from the histogram of sarcomere length in young muscles that one of the distribution peaks occurred at about this sarcomere length (Fig. 3).

The elongation of the sarcomeres to the adult value of approximately 2.8 μ took place by the pulling out of the individual sarcomeres as no change was found in the length of either the A- or I-filaments. This observation is consistent with recent evidence that the filaments are of a fixed and definite molecular assembly in vertebrate species (H. E. Huxley, 1963). The gradual pulling out or sliding of the thin over the thick filaments is presumably caused by the traction on the muscle fibres resulting from the lengthening of the bones to which they are attached via their tendons. It appears from the histogram in Fig. 3 that the rate of pulling out of the sarcomeres, and presumably the production of new sarcomeres in series, varies considerably even between young muscles of about the same age. Initially, considerable difficulty was encountered in trying to obtain with the electron microscope a true estimate of sarcomere length in very young muscles. Later it was realized that this was due to the difficulty of fixing the muscles at their maximum resting length (length at maximum limb extension). Great care was in fact necessary in pinning out the limb at the fully extended position because in young mice the elbow joint is readily dislocated with the result that the biceps muscle is stretched. As already described, this results in only a relatively small percentage of the sarcomeres in the middle of the muscle increasing in length by a considerable degree, thus giving rise to very substantial errors.
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It is well known that the initial length of the sarcomeres both at the middle and at the ends of the fibres has a marked effect on the tension the muscle can develop when activated (A. F. Huxley & Peachey, 1961). In this investigation it was found that the length/tension curves of young and mature muscles differed in their shape (Fig. 4). The tension developed by the young muscles showed a very strong dependence on the initial length, whereas the mature muscles showed much less dependence, particularly over the range of $-20$ to $+20$ per cent of the resting length. The reason for this is thought to be the fact that any change in the over-all length of the fibres of the young muscles affects only the middle 60% of the sarcomeres. In adult muscles the change in fibre length is spread over most of the sarcomeres. The mouse biceps muscle exhibited a surprisingly wide plateau in its length/tension curve. The reason for this may lie in the fact that the muscle is relatively bulky with oblique tendons at each end. Thus stretching the muscle may result in a change in the shape of the belly of the muscle (to be compared with the pivoting and change in shape of a parallelogram) in addition to a lengthening of the fibres. Another difference noted between the length/tension curves of young and mature muscles was that the young muscles did not develop their maximum strength until stretched to about 10% beyond their resting length. This is presumably because at the resting sarcomere length of the young muscles (2.3 $\mu$) any shortening would cause thin filaments to overlap and a disruption of the orderly arrangement of the filament lattice. Therefore it is only when the sarcomeres are pulled out slightly that they develop the maximum tension under the semi-isometric recording conditions used. The fact that the sarcomeres in the young muscles are already in the shortened state presumably means that the newborn animal is only able to flex its limbs in a relatively feeble fashion as further shortening of the sarcomeres involves a considerable drop in tension. The other implication of this work is that the longitudinal growth of the muscle fibre is restricted to an increase of not more than 20%, once the differentiation of new sarcomeres is complete.

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REFERENCES

Fig. 5. Examples of electron micrographs of sarcomeres from muscles of different ages. The body weight of the mice from which these muscles were taken were as follows: A, 49 g; B, 35 g; C, 24 g; D, 9 g; and E, 2.5 g. From this series it will be seen that the increase in sarcomere length during growth is due to a decrease in the overlap of the A-filaments by the I-filaments and not to a change in the length of either type of filament.