THE LENGTH–TENSION RELATIONSHIP OF THE DORSAL LONGITUDINAL MUSCLE OF A LEECH

BY JENNIFER B. MILLER

*School of Biological Sciences, University of East Anglia,
Norwich NOR 88C*

SUMMARY

The length–tension relationship of a preparation of the dorsal body wall of the leech *Haemopis sanguisuga* was determined.

Passive tension is low except at very long lengths of the preparation, when it rises steeply. It is due mainly to the epidermis present in the preparation.

The active tension curve is very flat, with tension being reduced only at very short and very long lengths. This shape is explained in the context of the myofilament arrangement of the muscle fibres. It may be that thin filaments can form cross-bridges with different thick filaments at different lengths of the preparation.

INTRODUCTION

The somatic muscles of annelids are of the oblique-striated or helical smooth type. There has been very little work on the mechanical properties of leech muscle, although it has been used for many years as a biological assay for acetylcholine solutions (see, for example, MacIntosh & Perry, 1950). There have been more experiments on the mechanical properties of earthworm muscle, but there is only one description of the length–tension relationship (Hidaka, Kuriyama & Yamamoto, 1969). The following account forms part of an investigation into the mechanical properties of the dorsal longitudinal muscle of the horse leech *Haemopis sanguisuga* (L.).

METHODS

Specimens of *Haemopis sanguisuga* were kept in aerated tanks of dechlorinated tap-water at 10–12 °C. The dorsal longitudinal muscle was prepared for experimentation by making an incision along the whole length of the animal on each side. Then, with the animal pinned out dorsal side uppermost, the dorsal wall was separated from the gut and connective tissue to provide a strip of the dorsal wall approximately 5 cm long by 0·5 cm wide. The strip was tied with cotton at each end and a small fish-hook, attached to a length of fine chain, was passed through the tissue and behind the cotton at each end of the preparation. The chains were used to mount the preparation on a vertical Perspex electrode assembly, with the lower end fixed and the upper end attached to a Devices Type 2STO2 strain gauge. The output from the strain gauge, which was proportional to tension, was amplified and displayed on a Devices M2 hot-wire pen-recorder.

The muscle was stimulated through an array of alternately positive and negativ◼ silver wires with 4 mm spacing, using a Grass S4 stimulator. The output from the stimulator was fed through an emitter-follower circuit so that Ringer solution of low resistance did not reduce the stimulus voltage. Between stimuli the muscle was immersed in Ringer solution of the following composition (mM): NaCl 113·0; KCl 4·3; $CaCl_2$ 2·0; $NaHCO_3$ 1·6; Na_2HPO_4 1·0; the pH being adjusted to 7·4 with 10% HCl (modified from Pantin, 1946). The experiments were carried out at room temperature (20–24 °C).

It has been shown that the leech dorsal longitudinal muscle responds with different types of contraction to different types of electrical stimulation, namely direct current and pulsed stimuli (Miller & Aidley, 1973). The problem of fatigue of this muscle tends to be somewhat greater with d.c. stimulation, and a full experiment to investigate the length–tension relationship involved a long period of experimentation and a large number of stimuli. Therefore, only pulsed stimuli were used in this investigation. A preliminary experiment had indicated that there were no parameters in the responses to pulsed and d.c. stimuli, such as rates of contraction and relaxation, which were affected differently by changes in length of the preparation. The pulsed stimuli consisted of a train of 5 msec square-wave pulses at a frequency of 50/sec for ½ sec, which is just sufficient for maximum tension to be reached. In all the experiments a check was made on possible fatigue by performing the readings in a cycle, enabling the first few points to be repeated at the end of the experiment. No significant fatigue occurred in any of the experiments.

For most preparations the total length change was between 2·5 and 3·5 cm. The length change between each set of readings was generally 4 mm. The starting length for most experiments was in the middle region of the range, but some experiments were started at very short or very long lengths. Measurements of the passive and active tensions were made at different lengths, having both increased and decreased the length of the preparation, so that the readings were made in a complete cycle, sometimes increasing the length first, and sometimes beginning by decreasing the length. Three contractions were made at each length.

The change in length of the muscle preparation was made at a steady rate (about 1 mm/sec) with the muscle immersed in Ringer solution to reduce any friction against the electrode. The immediate change in tension on altering the muscle length was always greater than the final tension change. Therefore after the initial tension increase caused by stretching the muscle, tension fell gradually to the new steady level. This phenomenon is known as stress relaxation. Conversely, on shortening the muscle the tension fell suddenly, but some tension was redeveloped extremely slowly, up to the final passive level characteristic of the muscle length.

THE STRUCTURE OF THE PREPARATION AND THE LONGITUDINAL MUSCLE

Any interpretation of the shape of the length–tension diagram must take into account the structure of the preparation itself, and the arrangement of the myofilaments in the longitudinal muscle which forms the principal constituent of the preparation. Therefore brief descriptions of both are included here.

The preparation

The preparation used in these experiments has already been described in detail (Miller & Aidley, 1973). It consists of part of the dorsal body wall of the leech, and therefore contains tissues other than the longitudinal muscles being investigated. The epidermis, the connective tissue and the spongy inner layer of botryoidal tissue are all passive tissues, which make no contribution to the active development of tension, though they will certainly affect the passive tension. There are three layers of muscle cells, namely circular, longitudinal and oblique muscle. The circular muscle, being perpendicular to the direction of tension measurement, will not affect the tension developed by the longitudinal muscles, but the small number of obliquely orientated muscle fibres will do so. It has been estimated (Miller & Aidley, 1973) that their contribution does not exceed 10% of the maximum tension developed by the longitudinal muscle fibres, and will generally be much less than this. However their contribution, though small, will depend on the length of the preparation. The angle they make with the longitudinal axis of the preparation (i.e. the direction of tension measurement) will decrease with increasing length of the preparation. Thus their contribution, being equal to the cosine of this angle, will increase. Attempts were made to measure accurately the angle of the oblique fibres by phase contrast and polarized light microscopy, but this was found to be almost impossible because of the presence of the botryoidal tissue, which was very difficult to remove cleanly and completely. It was quite impractical to attempt this for each of the preparations used in the length–tension experiments. The angle is estimated to be of the order of 10–25°, depending on the length of the preparation.

The longitudinal muscle

As already explained, the muscles of the leech are of the oblique-striated type. The muscle cell has the form of a long tapering cylinder, with the contractile material forming a peripheral cortex. Mill & Knapp (1970) have described the myofilament arrangement in earthworm muscle, and the picture appears to be very similar for leech muscle. Fig. 1 shows a very simplified diagram of the myofilament arrangement in a block of oblique-striated muscle, based on their description. A short electron-microscope study of the longitudinal muscle cells of *Haemopis sanguisuga* (see Miller, 1974) has indicated that the arrangement in the horse leech is basically the same as it is in earthworm muscles and in the muscles of other leeches (Röhlich, 1962; Pucci & Afzelius, 1962).

RESULTS

All the preparations showed the same type of length–tension relationship, and Fig. 2 shows the results of a typical experiment.

Passive tension

Over most of the length range there was a very low level of passive tension, and this was affected little by the length of the preparation, increasing only slightly as the length was increased. However, at long lengths of the preparation the passive tension
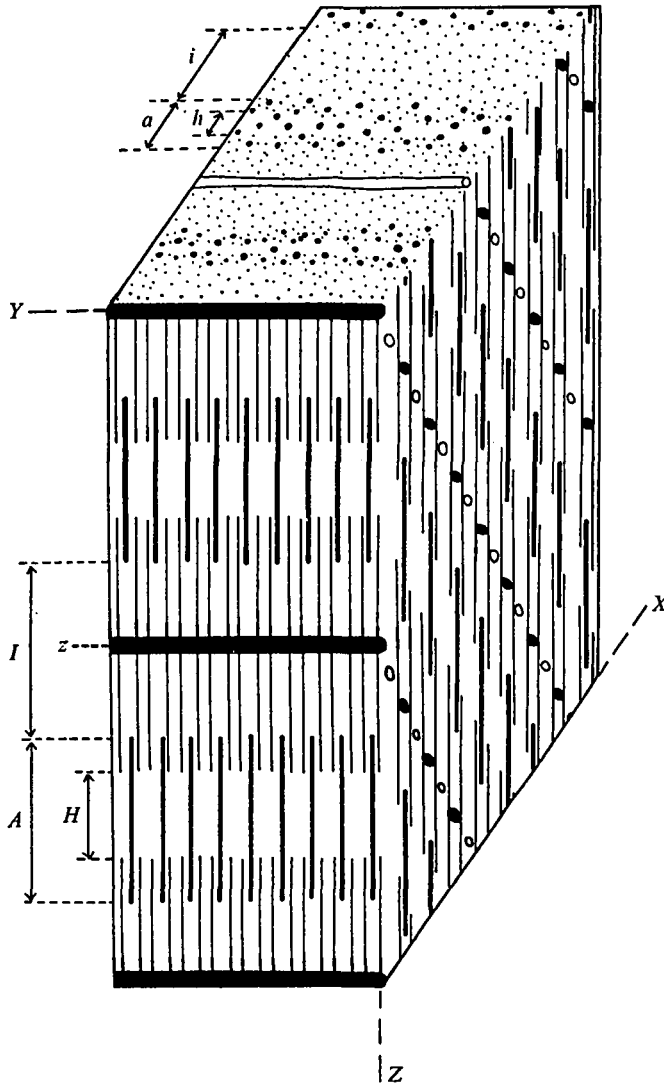
Fig. 1. Diagrammatic representation of a block from an obliquely striated muscle fibre. X, Y and Z are the three perpendicular axes by which the transverse (xy), the radial longitudinal (yz) and the tangential longitudinal (xz) planes are defined. The angle of the striations in the xz plane is exaggerated for clarity. The Z-rods (z) are shown as solid rods, and the transverse tubules of the sarcoplasmic reticulum as hollow rods. The thick and thin filaments are shown as thick and thin vertical lines. The A, I and H bands of the sarcomere are denoted by A, I and H respectively when seen in longitudinal section, and by a, i and h respectively in cross-section. After Mill & Knapp (1970).

increased very rapidly with increasing length to a very high tension compared with the maximum active tension. This is shown by the very steep region of the passive tension curve in Fig. 2.

Active tension

The active tension showed a very flat curve, with no obvious symmetrical peak as is found in cross-striated muscle. At any particular length there was the normal small variation in peak tension developed by the muscle in the series of three contractions.
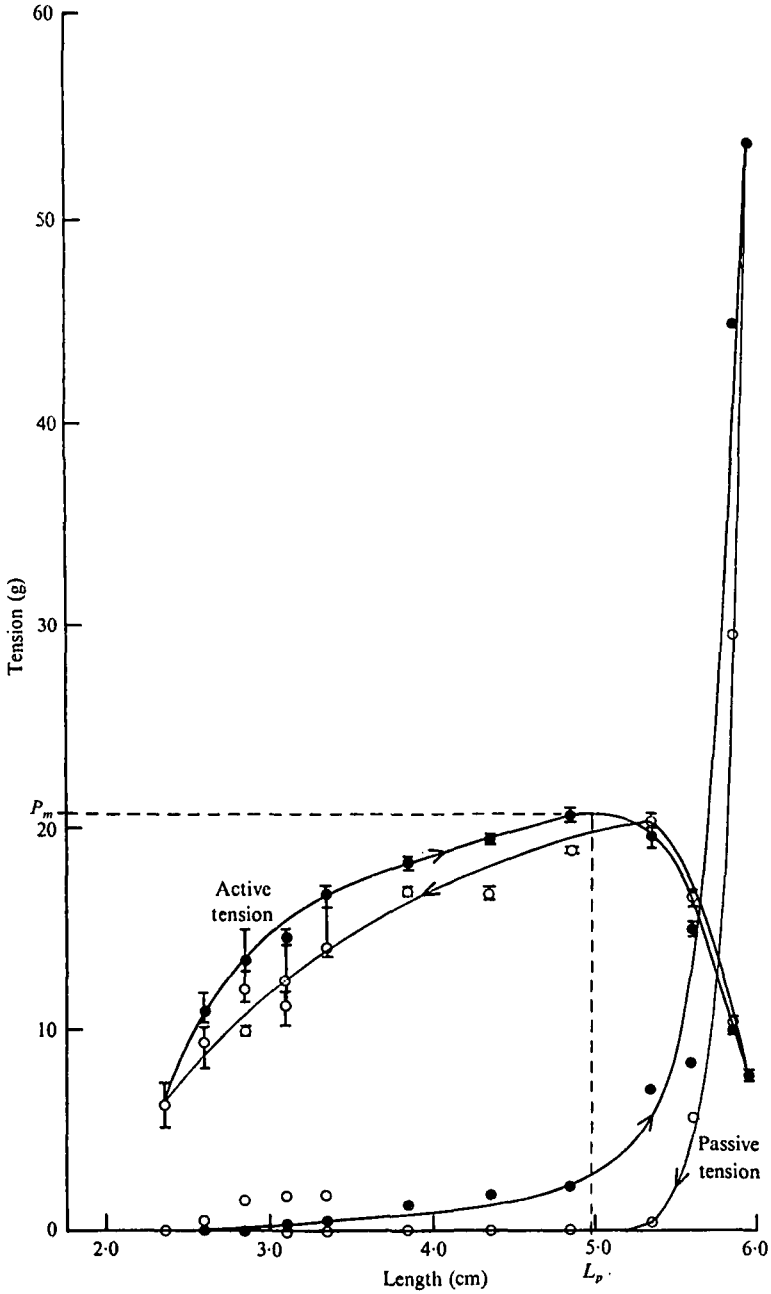
Fig. 2. Length–tension relationship of the leech dorsal longitudinal muscle. The points for the active tension (i.e. the increase in tension on stimulation) represent the mean and range of three values. The arrows on the curves indicate the order in which the readings were taken, the preparation's length being either increased (●) or decreased (○) to reach the new length. L_p and P_m, as defined in the text, are indicated on the diagram.

This is indicated in Fig. 2 by a vertical bar showing the range of values covered by the three points. Over the range of lengths in which the passive tension was small and fairly constant, the active tension increased very gradually with increasing length in an almost linear fashion. The peak of the curve was at approximately that length at which the steep passive tension increase started to occur, and then the fall-off in active tension was very marked as the length of the preparation was increased. At this stage the muscle was very severely stretched, being probably $2–2\frac{1}{2}$ times the length at which the minimum readings were taken. There was also a rapid decline in active tension at very short lengths. This decline occurred at such short lengths that the muscle was quite slack.

Hysteresis

Hysteresis occurred in both the passive and active tension curves. If at any stage during stretching the length of the preparation was reduced, the passive tension dropped far more than would be predicted from the curve of ascending readings. This hysteresis could not be explained by any damage to the muscle caused by the severe stretch, since if the cycle were repeated the ascending readings followed the upper curve and the descending readings the lower one. Because of the very high passive tensions encountered at long muscle lengths, each preparation was carefully examined at the end of the experiment to see whether there was any damage to the tissue at the point of insertion of the fish-hook. None was found.

For the active tension, the ascending readings were higher than the descending readings over the range of lengths covered by the more or less linear region of the curve. At longer lengths, where the fall-off in active tension occurred, the greater active tension was developed when the muscle was shortened prior to the readings. There was, therefore, a cross-over in the two curves at the length at which the peak active tension occurred. The curve for the descending readings resembled that for the ascending readings, but displaced to the right.

Comparison between different preparations

An attempt was made to standardize the values obtained from different preparations. For most muscles it is possible to define fairly easily a 'reference length' or 'L_o' value, either by the length of the muscle in the body, or by its resting length, or by the length at which passive tension begins to be present. For the leech muscle preparation there is no obvious 'resting length'. The body length of the leech can vary as much as the length of the muscle preparation even after death. There is a wide range of lengths of the preparation over which passive tension is present, though it is very small and to a large extent independent of the length.

Therefore, in order to compare different preparations, the reference point on the length scale was chosen as the length at which the maximum active tension occurred (on the curve for values obtained when increasing the length of the preparation). This value was called L_p. The reference point on the tension scale was taken as the maximum active tension, called P_m, also using the curve for readings taken after a length increase. The mean values of tension and length for each preparation were then expressed as fractions of P_m and L_p respectively. Fig. 3 shows the results of these
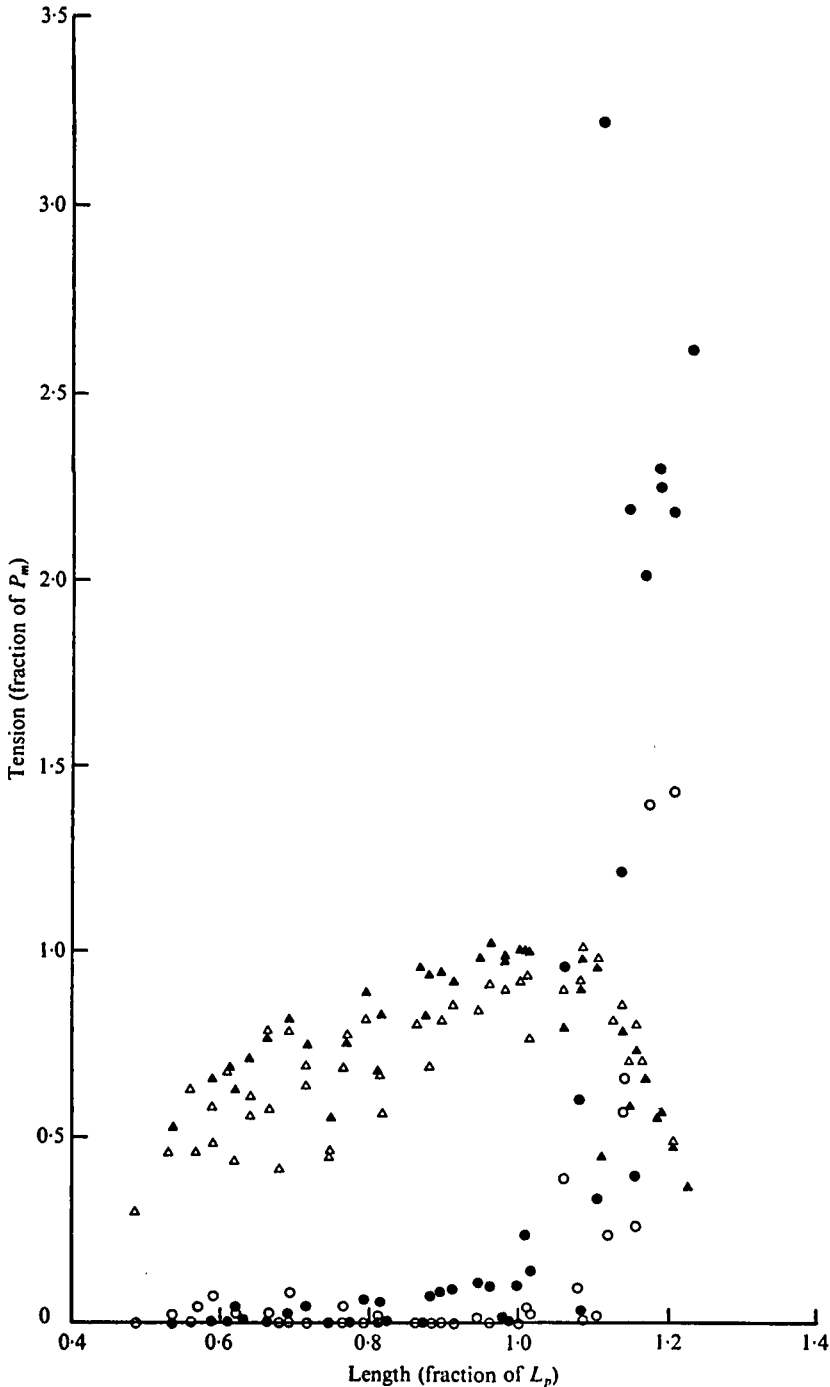
Fig. 3. Length–tension relationship of the leech dorsal longitudinal muscle, using data from four preparations. The tension is expressed as a fraction of P_m, the maximum active isometric tension for each preparation, and the length as a fraction of L_p, the length at which P_m occurs. Open symbols indicate that the length was reduced prior to the reading, and filled symbols that the length was increased. Each point for the active tension (triangles) represents the mean of three values. Circles represent the passive tension.

4

calculations for four preparations, and the passive tension curve clearly shows th
hysteresis already described. The hysteresis in the active tension is less marked, being
masked to a certain extent by the variability in tension between preparations.

DISCUSSION

The length–tension diagram of the leech muscle obtained in these experiments
appears to be very similar to that obtained for earthworm longitudinal muscle by
Hidaka *et al.* (1969), except that the fall-off in active tension at short lengths is more
gradual in the earthworm. The leech diagram differs from that of cross-striated
muscle in several ways.

Passive tension

The level of the passive tension is quite low until the muscle is considerably
stretched, and then the rise of passive tension as the length is increased is very rapid.
Cross-striated muscle, in which the parallel elastic component consists mainly of the
muscle fibre membranes and connective tissue, has a passive length–tension relation-
ship in which tension develops as soon as the body length is exceeded. The parallel
elastic component affects the tension level over the whole length range above L_o, and
it is fairly compliant. The difference between such a muscle and the leech dorsal
muscle is due to the fact that in the latter the parallel elastic component, which con-
sists principally of the epidermis, is relatively inextensible, so that when it is stretched
the rise in tension is rapid. However, it is long compared with the normal length of
the preparation, and at short lengths it is able to wrinkle (this is indicated in a pre-
paration at short length by the prominence of the annuli, into which the segments of
the leech are divided). Hence it does not begin to affect the passive tension until the
epidermis is fully extended.

Active tension

It has been suggested by Rosenbluth (1967) that length and tension could be dis-
sociated in oblique-striated muscle because there may be two contraction mechanisms,
namely sliding and shearing. ('Sliding' refers to a change in the amount of overlap
between thick and thin filaments, as in cross-striated muscle; 'shearing' refers to a
sliding between adjacent thick filaments, thus changing the angle of the oblique
striations.) Knapp & Mill (1971) also presented evidence for the existence of these
two mechanisms of contraction. The flat form of the active length–tension curve over
a wide range of lengths might seem at first sight to support Rosenbluth's hypothesis
that length and tension can be dissociated, but these results show that there is in fact
a very precise relationship between the active tension developed by the muscle and
its length. This is supported by the fact that data from different preparations are in
close agreement (see Fig. 3) and also by the similarity of the curve for the earthworm
muscle (see Hidaka *et al.* 1969). Lanzavecchia (1968) has produced some very strong
geometrical arguments against Rosenbluth's hypothesis, and it will now be seen that
consideration of the myofilament structure of this muscle can provide a reasonable
explanation for the shape of the length–tension diagram without the need to propose
any dissociation of length and tension.

If an oblique-striated muscle is observed at high magnification in cross-section, it

▶an be seen that although the arrangement of the thick filaments is fairly regular, there is little regularity about the arrangement of the thin filaments as in, for instance, insect flight muscle (see, for example, Mill & Knapp, 1970; Miller, 1974). The position of a thin filament relative to the nearby thick ones is probably fairly flexible. At very long lengths of the muscle preparation it is possible to visualize a situation where there is no overlap between a particular thin filament and the thick filament with which at shorter lengths it formed cross-bridges. In cross-striated muscle, with all the filaments in register across the muscle fibre, this would occur at the same length with all the thick and thin filaments. Formation of cross-bridges would then be impossible and active tension would be zero. With oblique-striated muscle, however, when the muscle is extended sufficiently to eliminate overlap between the thin filament and the thick filament with which it had been forming cross-bridges, there would still be some overlap present between that thin filament and a nearby thick one. This occurs because adjacent thick filaments in an *A* band are longitudinally displaced. The formation of cross-bridges between the new 'partners' might involve a small lateral displacement of the thin filament, but this would not appear to be impossible in view of the rather random arrangement found among the thin filaments.

This hypothesis could explain the very large length range over which the muscle can operate. It could also account for the very smooth flat nature of the active length–tension curve, since the active tension would depend on the total amount of overlap found in a whole population of myofilaments in which the overlap between any two filaments varies continuously over the whole range of lengths of the preparation. It would not be until the extremely long lengths were encountered that all overlap would be abolished. Similarly, only at very short lengths of the preparation would the thick filaments be bunched up against the *Z*-lines, particularly as they are in the form of one-dimensional rods rather than two-dimensional plates. Knapp & Mill (1971), who studied earthworm muscle fixed at different lengths, stated that they observed no thick filaments projecting beyond the *Z*-lines into the next sarcomere, but this possibility is not necessarily excluded. The stimulation period of 500 msec is only just sufficient for the muscle to develop maximum tension. Longer periods of stimulation were avoided because of the problem of fatigue. If the peak tension were not quite reached within the 500 msec, then at short lengths, when the muscle is slack, the time taken for the slack to be taken up before tension is generated would be a significant factor, leading to an artificially low value for the active tension at very short lengths. It might be that the fall-off in active tension at very short lengths would be slightly less steep with a longer stimulation period.

The hypothesis of 'changing partners' between the thick and thin myofilaments as the length of the muscle changes might also explain the hysteresis in the active length–tension curve. Considering the rather random arrangement of the thin filaments there is no reason for every thin filament to change its 'partner' in cross-bridge formation at precisely the same length when the muscle is being shortened as when it is being extended. However, this hypothesis is unlikely to account for the very consistent nature of the hysteresis found in all the preparations on which measurements were made of the length–tension relationship. We would perhaps expect the changing of the filament 'partners' to occur in a more random fashion.
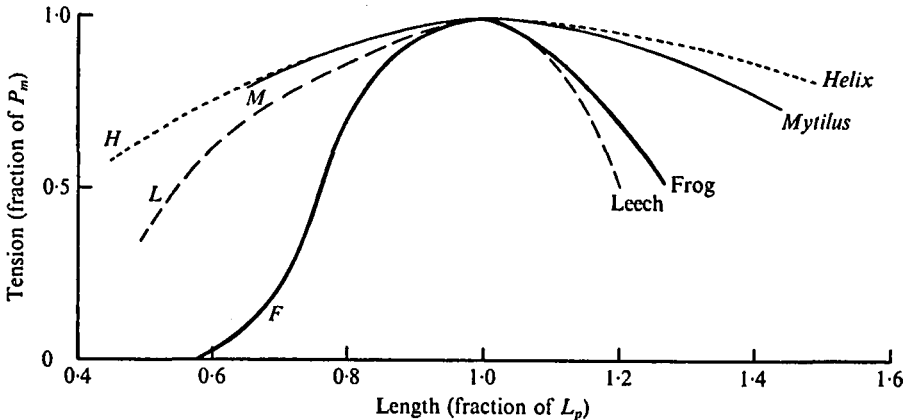
Fig. 4. Active length–tension curves for *Mytilus* (A.B.R.M.), *Helix* (pharynx retractor muscle) and the frog (sartorius muscle) modified from Csapo (1960). The curves are plotted on the same basis as that for the leech (see text for details), which is included for comparison.

The oblique muscle fibres

The contribution of the obliquely orientated fibres does not exceed 10% of the tension developed by the longitudinal fibres, though it is slightly greater at longer lengths. It is possible, therefore, that the reduction in the contribution of the oblique fibres may account for at least some of the gradual decrease in the active tension as the length of the preparation is reduced below L_p. But it cannot account for the sudden reduction of active tension at very long lengths of the preparation, since it is under these conditions that the contribution of the oblique fibres should theoretically be greatest. Considering the small quantity of oblique fibres compared with the longitudinal fibres, it is probably fair to assume that the length–tension relationships shown here are a true reflexion of the length–tension relationship of the longitudinal muscle, although the shape may be very slightly modified by the oblique fibres. The fact that the length–tension curve of the leech is qualitatively similar to that of the earthworm, which has no oblique fibres, would seem to confirm this.

Comparison with other types of muscle

It is interesting to compare the shape of the leech length–tension curve with that for other species. Fig. 4 shows active tension curves for *Mytilus* (anterior byssus retractor muscle), *Helix* (pharynx retractor muscle) and the frog (whole sartorius muscle). The curves are taken from Csapo (1960) but replotted on the same basis as is used for the leech muscle preparations, calculating tension as a fraction of P_m, the maximum active tension, and length as a fraction of L_p, the length at which P_m occurs. The curve for leech muscle obtained in this study is also included. The flatness of the leech curve has been ascribed to the oblique, rather than transverse registering of the thick filaments. In the smooth muscles of *Mytilus* and *Helix* the thick filaments are completely unregistered, and we might expect this to be an even more continuous system than is found in oblique-striated muscle. The curves are seen to be correspondingly even more flat, with no rapid fall-off of tension even at long lengths.

The active tension in the leech muscle falls off more rapidly at very long lengths than it does even in the frog sartorius muscle. This, of course, has a more symmetrical

rve and a more prominent peak than the leech. Theoretically the fall-off of tension at long lengths occurs when no overlap remains between the thick and thin filaments. It is difficult to see how this could occur more rapidly in a population of oblique-striated muscle fibres than in a population of cross-striated muscle fibres, in which the thick filaments are fully in register.

A satisfactory explanation of the basic features of both the active and passive length–tension curves of the leech muscle can thus be given by considering the structure of the preparation and of the longitudinal muscle itself. It is not necessary to postulate that there are two contraction mechanisms, or that length and tension can be dissociated. Rather, we are dealing with a muscle in which tension can be developed over a wide range of lengths because of the particular structure of the muscle fibres.

REFERENCES

CSAPO, A. (1960). In *Structure and Function of Muscle*, vol. 1 (ed. G. Bourne). New York: Academic Press.

HIDAKA, T., KURIYAMA, H. & YAMAMOTO, T. (1969). The mechanical properties of the longitudinal muscle in the earthworm. *J. exp. Biol.* **50**, 431–43.

KNAPP, M. F. & MILL, P. J. (1971). The contractile mechanism in obliquely striated body wall muscle of the earthworm, *Lumbricus terrestris. J. Cell Sci.* **8**, 413–25.

LANZAVECCHIA, G. (1968). Studi sulla muscolatura elicoidale e paramiosinica. II. Meccanismo di contra-zione dei muscoli elicoidali. *Atti Accad. naz. Lincei Rc.* **44**, 575–83.

MACINTOSH, F. C. & PERRY, W. L. M. (1950). Biological estimation of acetylcholine. In *Methods in Medical Research*, **3**, 78–92 (ed. R. W. Gerard). Chicago: Yearbook Publishers Inc.

MILL, P. J. & KNAPP, M. F. (1970). The fine structure of obliquely striated body wall muscles in the earthworm, *Lumbricus terrestris* Linn. *J. Cell Sci.* **7**, 233–61.

MILLER, J. B. (1974). *Mechanical Properties of a Leech Muscle*. Ph.D. Thesis, University of East Anglia.

MILLER, J. B. & AIDLEY, D. J. (1973). Two rates of relaxation in the dorsal longitudinal muscle of a leech. *J. exp. Biol.* **58**, 91–103.

PANTIN, C. F. A. (1946). *Notes on Microscopical Technique for Zoologists*. Cambridge University Press.

PUCCI, I. & AFZELIUS, B. A. (1962). An electron microscope study of sarcotubules and related structures in the leech muscle. *J. Ultrastruct. Res.* **7**, 210–24.

RÖHLICH, P. (1962). The fine structure of the muscle fiber of the leech *Hirudo medicinalis. J. Ultrastruct. Res.* **7**, 399–408.

ROSENBLUTH, J. (1967). Obliquely striated muscle. III. Contraction mechanism of *Ascaris* body muscle. *J. Cell Biol.* **34**, 15–33.