ELONGATION OF CROSS-STRIATED MYOFIBRILS

by

JEAN HANSON

Medical Research Council Biophysics Research Unit, King's College, London (England)

After a muscle has contracted it relaxes and eventually returns to rest length; as the "active state"[1] decays, extensibility is restored, and it would then be possible for an antagonistic muscle to pull the shortened muscle back to its original length without its active participation in the process. There is indeed much evidence[2] that lengthening is entirely passive and that contraction is the active part of the cycle. An observation made recently during studies on the indirect flight muscles of the blow-fly *Calliphora* shows, however, that the shortened sarcomeres of an isolated myofibril can re-extend themselves. The photographs in Fig. 1 show a short piece of an isolated fibril, consisting of only twelve sarcomeres, undergoing cycles of elongation and contraction. In Fig. 1a the total length of all the sarcomeres is 40 μ. It has increased by 4% in 1b, and an H zone and imperfectly-resolved I bands have appeared in some of the sarcomeres; in all of them the CM lines have disappeared and the CZ lines have become much thinner. In Fig. 1c each sarcomere has shortened again and has nearly resumed its original appearance, but in 1d the fibril has once more lengthened and its cross-striations have altered. These small changes in length in individual sarcomeres (less than 0.2 μ) cannot be measured accurately, but the total change in all twelve sarcomeres is 1.6 μ. The striking reversible alterations in the pattern of the cross-striation (seen particularly clearly in Fig. 3) were the first indication, until photographs could be obtained, that the fibrils were lengthening and shortening, for studies on both rabbits[3] and insects had already shown that the H zones and I bands disappear during contraction; their reappearance suggested elongation. These reversible changes in sarcomere length were obtained by irrigating preparations of fibrils under the microscope with a crude suspension of sarcosomes from the same muscle. The fibrils lengthened while irrigation was in progress and shortened when it ceased; when it was resumed they lengthened again.

MATERIAL AND METHODS

Blow-fly larvae (*Calliphora erythrocephala* Mg. and *C. vomitoria* L.) were obtained from a dealer; both species gave the same results. After the flies had emerged they were fed on raw meat and sugar and were used during the second or third weeks of adult life. The thorax was cut out of a living fly and bisected longitudinally, and the indirect flight muscles were put into a simple salt solution consisting of 0.1 *M* potassium chloride and 0.0067 *M* Sörensen phosphate buffer at pH 7.0. Preparations of isolated fibrils were made by teasing a small group of muscle fibres very gently with fine needles in a drop of salt solution on a microscope slide. Suspensions of sarcosomes were made by roughly teasing the muscles in a small quantity of salt solution, about 4 ml being used for all the indirect flight muscles of one fly. These concentrated sarcosome suspensions were used without dilution; neither calcium nor magnesium was added to the system. No attempt has yet been made to discover what factors in the crude sarcosome suspensions are responsible

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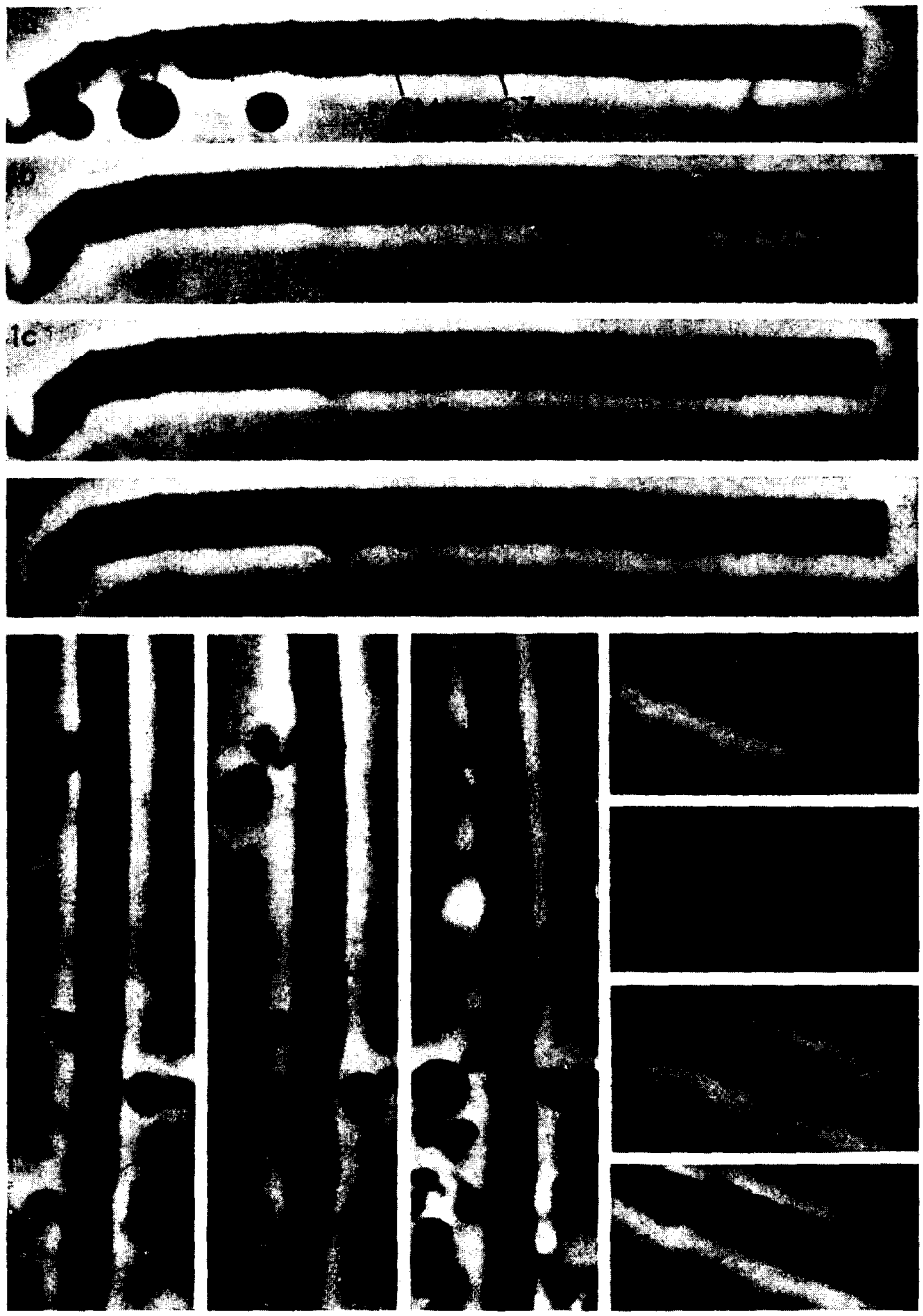

Photographs taken in a phase contrast microscope of three myofibrils isolated from the indirect flight muscles of *Calliphora*. Magnification 2800 ×. Figs. 1a, 2a and 3a show three untreated fibrils. The elongation of the sarcomeres observed in Figs. 1b, 2b and 3b was brought about by treatment with a crude suspension of sarcosomes. When treatment ceased (Figs. 1c, 2c and 3c) the sarcomeres contracted, and when it was resumed (Figs. 1d and 3d) they elongated again.

for elongation or contraction. It has been found that only freshly-prepared suspensions will make the fibrils lengthen; after they had been kept for about forty-five minutes at room temperature they immediately induced contraction. In a few experiments in which the sarcosome suspensions were made from recently-emerged flies, it was found that they always induced contraction, perhaps because the sarcosomes were not yet fully grown[4]. Even when older flies were used for preparing the sarcosome suspensions the fibrils sometimes failed to elongate before they contracted; this is not surprising in view of the complexity of the system.

In the short fibril illustrated in Fig. 1 all twelve sarcomeres lengthened simultaneously and none of them was stretched as a result of contraction in another part of the fibril. A long fibril behaves in the same way; and if its movement is restricted it becomes slightly folded (Fig. 2) or moves downwards away from the coverslip between the points of attachment; this again shows that the elongating sarcomeres are not being stretched. This point was also demonstrated by a different experiment. A suspension of completely isolated fibrils and sarcosomes in saline was prepared and samples examined under the microscope; nearly all the fibrils were of the type illustrated in Fig. 1a, and very few of them showed H zones or I bands. This suspension was then mixed with an equal volume of glycerol and left in the refrigerator overnight. The next day samples were examined and it was found that every fibril had acquired H zones and I bands; these fibrils could then be made to contract and resume their original appearance. It is useless to quote sarcomere measurements in this last experiment, for it has been found that in these insect muscles two fibrils of the same band pattern may have different sarcomere lengths; in any individual fibril, however, length and pattern are strictly correlated.

It is not proposed in this brief report to discuss what are the factors which might be responsible for elongation and for contraction, although it may be supposed that in these insect muscles, as apparently in rabbit muscles[5], the concentration of adenosine triphosphate inside the fibril and the level of myosin adenosinetriphosphatase activity may control the system. The purpose of publishing these results is to point out that myofibrils isolated from the indirect flight muscles of *Calliphora* are capable of active elongation. (The word "active" used in this context does *not* imply that relaxation is the phase of the contraction-relaxation cycle in which energy is used.) These flight muscles, which effect wing movements by deforming the thoracic exoskeleton, contract under nearly isometric conditions[6], and the percentage change in sarcomere length shown by isolated fibrils is probably about the same as (or slightly more than) the percentage change in muscle length during flight. Experiments which will be described in another paper indicate that fibrils *in situ* have the same band patterns as those in the photographs published here. It is possible that active elongation plays some part in the normal functioning of these muscles; PRINGLE's studies[7] on the tymbal muscles of a cicada, which function in a similar manner, have thrown new light on this subject. This type of muscle differs in many respects from the more familiar skeletal muscles of vertebrate animals, and it does not necessarily follow from the present observations that an isolated myofibril from a vertebrate skeletal muscle can re-extend itself. An unloaded isolated vertebrate muscle fibre is able to lengthen after it has contracted[8], but this may be attributable to elastic recoil of the deformed connective tissue sheath[2]. It seems doubtful, however, if such lengthening of the fibres plays any part in normal functioning, for HILL[2] has demonstrated that an unloaded intact muscle remains at the length it assumes on con-

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traction; this suggests that the muscle in the body relies entirely on its antagonist to restore it to rest length. In spite of this, there is some inconclusive evidence that the myofibrils in vertebrate skeletal muscles may lengthen when conditions permit. MARSH[9] discovered that small pieces of homogenised rabbit muscle, in the presence of adenosine triphosphate, could elongate by as much as 40% under the influence of the Marsh-Bendall factor (myokinase[10]); without the factor they contracted. It seems likely that the sarcolemma in these fragmented fibres was too damaged to have caused this elongation, and it may have been brought about by the fibrils themselves. In a film made by HARMAN[11] of the behaviour of fragments of muscle (pigeon breast, rat diaphragm, rabbit psoas) while their sarcoplasmic components were engaged in oxidative phosphorylation, alternating elongation and contraction in the myofibrils was convincingly demonstrated; in an earlier publication[12] the same phenomenon was reported. However, it is quite possible that in this system the elongating sarcomeres were being stretched by other sarcomeres which were contracting; the fibrils in the field of view did not all behave synchronously.

The isolated insect myofibrils in which active elongation has been described do not possess any surface membrane[13] that could recoil to its original configuration at the end of contraction, moving the unresisting contractile components back with it. On the other hand, it has not been demonstrated that any structures in the fibril are lengthening by an energy-consuming process. It is much more likely that the phenomenon we have been considering takes place in two stages, firstly an "unlocking" (by adenosine triphosphate, for example) of the contractile elements[3] which had been arrested in the shortened state, and secondly the passive return to rest length of different elements, also inside the fibril, which had been deformed during shortening; these could be in series with or parallel to the contractile elements. One can speculate that the passive component might be the stroma which remains in the form of a continuous "backbone" after other materials including actomyosin have been extracted from the fibril.

SUMMARY

When myofibrils isolated from the indirect flight muscles of *Calliphora* were treated under the microscope with freshly-prepared crude suspensions of sarcosomes from the same muscles they elongated by about 4% and then contracted when treatment ceased; several cycles of elongation and contraction could be brought about. Evidence is given that the elongating sarcomeres are not being passively stretched, but are themselves extending, and the implications of this finding are discussed.

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