Note added in proof. Since submitting this article we have learnt that Drs. H. E. Huxley, W. Brown and K. C. Holmes, using frog sartorius muscle and an essentially similar method, have shown that the spacings and intensities of the '59' Å actin reflexion, and the '72' and '144' Å myosin reflexions, do not change during contraction (short tetanus). The results we have now also support their finding that the intensity distributed along the layer lines decreases relative to the meridional reflexions in contracting as compared with resting muscle.

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Constancy of Axial Spacings in Frog Sartorius Muscle during Contraction

Striated muscles give a characteristic system of low-angle X-ray reflexions. These reflexions can be seen using preparations maintained in a normal physiological condition and still able to contract[1,2]. A number of axial reflexions can be observed in addition to the equatorial reflexions which arise from the hexagonal lattices of overlapping actin and myosin filaments[1,3]. Part of the axial pattern comes from the actin filaments and closely resembles the patterns given by oriented samples of purified actin[4–6]; in muscle, the most prominent feature here is a strong off-meridional reflexion at 59 Å. At smaller angles a number of reflexions corresponding to an axial period of approximately 435 Å are observed, and Worthington[7], whose measurements are more accurate than the original ones[1], has given good reasons for believing that they come from the myosin filaments. According to the sliding-filament model, passive changes in the length of the muscle are not accompanied by any changes in the lengths of either type of filaments, and so the original observation[1] that all the axial reflexions are unchanged by passive stretch is precisely what we should expect.

It is of great importance for theories of contraction to know whether changes in the internal configuration of the actin or myosin filaments occur between the resting and the active state of the muscle. Much information from electron-microscopy indicates that overall changes in filament lengths do not occur[8,9]; but more detailed evidence obtained from X-ray diffraction experiments in which the muscle can be preserved in its living state throughout would be most welcome. However, even the most rapid low-angle diffraction systems have previously taken several hours to register the axial spacings from surviving muscles; such exposure times obviously make it extremely difficult to examine actively contracting muscles. We now find that the pattern can be recorded in as little as 10–20 min using a slightly modified version of a focusing quartz-monochromator and fine-focus rotating anode tube combination, recently introduced by one of us (K.C.H.).

Sartorius muscles dissected with extreme care from frogs in prime condition were maintained at 3°–5° C in oxygenated Ringer's solution fortified with metabolites. They continued to give good contractions for periods as long as 40–50 h in some cases, being stimulated electrically to give 1-sec tetani at 1-min intervals, by supra-maximal pulses of width 2–10 msec and repetition frequency 10–16 per sec. This gives total available exposure times up to 40–50 min, although our pictures were obtained in times shorter than this. Muscles, the endurance of which was less satisfactory, as was sometimes the case, could be replaced on the camera by fresh specimens and the exposure continued. The muscles were attached to an isometric lever and gave tensions up to about 60 g (equivalent to 2–3 kg/cm² of their cross-section); the average tensions during the experiment were about 80 per cent or more of the starting tension. A shutter was arranged so that the diffraction pattern was recorded only during that portion of each tetanus when the externally measured tension exceeded a certain value, usually about 30 g. Control diffraction patterns were recorded before and after the long series of short tetani, and these were found to be normal. Experimental details will be given more fully elsewhere.

Table 1. Measurements of Axial Spacings in Frog Sartorius Muscle

	Myosin reflexions (Å)		Actin reflexion (Å)
Relaxed	144·8 ± 0·4	72·8 ± 0·4	60·8 ± 0·1
Contracted	145·5 ± 0·8	73·3 ± 0·4	59·95 ± 0·2

We find that, to the limits of accuracy of our measurements (about 1 per cent normally, but as little as 0·5 per cent in the case of some measurements of the myosin spacings) the spacing of both sets of axial reflexions remained unaltered when the muscles contracted (Table 1). No major changes in the relative intensities of the strongest reflexions were observed and no prominent new reflexions appeared either. The experiments show, therefore, that no changes in the repeating periodicities (and hence in the overall lengths) of the major part of the actin and myosin filaments occur when the muscle becomes fully active and develops isometric tension; and that probably no major conformational changes occur synchronously within all units of these repeating patterns. This by no means rules out, however, the possibility that local changes in periodicity or in configuration or in both, may occur during contraction, restricted at a given instant to a short region of the filament but changing in position with time.

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Since making these observations we have learned that Drs. G. Elliott, J. Lowy and B. Millman, who have pioneered the study of the equatorial reflexions during contraction, have obtained similar results using toad sartorius muscle (preceding communication).

Note added in proof. We have now obtained diagrams, using a camera with high resolving power in two directions, which show a large decrease, during contraction, in the intensities of the off-meridional portions of the myosin layer-lines (which are themselves weaker than the meridional myosin reflexions discussed here), indicating a movement of the cross-bridges.

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Sub-structure of Quantasomes

The protein layer in the lipoprotein lamellae of chloroplasts must be considered as an aggregation of two-dimensional crystallites, each one consisting of 16–32 lattice points. The crystallites can either be arranged regularly