ESRF	**Experiment title:** HIGH RESOLUTION X-RAY DIFFRACTION OF CONTRACTING MUSCLE	**Experiment number:** LS-468
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Report: The experiments we proposed were to carry out high resolution X-ray diffraction studies of muscles of muscles undergoing various forms of contraction with the objectives to establish: A) the extent of thick and thin filament extensibility; B) the extent of changes in the helical symmetry of the thin filaments, and; C) what fraction of the various spacing changes occurring in the myosin based meridional reflections is due to either thick filament extensibility or to the formation of an actomyosin (AM) complex. We are pleased to report that these objectives have been fully accomplished. The summary is:

A. The brilliance of the ESRF allowed very accurate measurements of the spacings of the actin and myosin diffraction features. A summary of the results is given in Tables 1, 2 and 3. As regards actin (Tables 1 and 2) we find that whether one uses the spacings of the ca. 5.9 and 5.1 nm layer lines to deduce length changes of the actin filament (F-actin) or the direct measure given by the ca. 2.7 nm layer line, the actin filament shortens by at least 0.42% as a result of a quick release sufficient to reduce tension from the plateau of isometric contraction (P_o) to a negligible value. As regards myosin, we were able to resolve and measure the spacings of the interference peaks splitting the meridian of the third (3M) and sixth (6M) layer lines at Po (Table 3). This allowed us to demonstrate that in a quick release there is a reduction in the interference distance very similar to the length change undergone by F-actin;

B. Straightforward application of helical diffraction theory to the data (Tables 1 and 2) demonstrates that, in addition, F-actin also changes is helical symmetry by untwisting during the transition form rest to Po and over-twisting relative to the rest state during the quick release. Thus, it appears that the thin filament is not only more extensible but also much more active than hitherto believed, and;

C. Finally, the data shows that the formation of an AM complex accounts for 1.45- 1.55%

of the spacing change of the 3M and 6M during isometric contraction, whilst a 0.6-0.8% change in interference distance occurs during the release. This implies a length change of the myosin diffraction units of 0.36-0.48%/half sarcomere, i.e comparable in magnitude to that of the thin filament.

The major conclusion we can report here is that the data shows that all the sarcomere elasticity in contracting muscle can be explained by filament extensibility. This means that the attached myosin heads in the AM complex formed during contraction are much stiffer than currently believed, a result which has profound implications for most current models of contraction.

Table 1

Spacings (in nm) of actin layer lines at:	REST	PLATEAU	RELEASE
37.0 (i)	-	37.156±0.205	-
5.9	5.909±0.004	5.923±0.006	5.903±0.004
5.1	5.087±0.003	5.105±0.005	5.071±0.007
37.0 (ii)	36.606±0.125	36.934±0.109	35.995±0.221
2.7 (ii)	2.734±0.002	2.742±0.003	2.728±0.003
N	8	11	6
2.7 (i)	2.734±0.001	2.742±0.001	2.730±0.001
N	6	10	8

Notes: (i) Spacings measured directly from diffraction data: (ii) Spacings deduced from measured spacings of the 5.9 and 5.1 layer lines. N here and in all other tables is the number of muscles used in the measurements. Error here and in all other tables is given in standard deviations.

Table 2

layer line	PLATEAU(*)	RELEASE(*)	RELEASE(**)
5.9	0.25±0.10	-0.10±0.07	-0.35±0.07
5.1	0.34±0.11	-0.32±0.13	-0.66±0.13
D(5.1-5.9)(i)	0.09±0.04	-0.22±0.10	-0.32±0.08
2.7 (ii)	0.30±0.10	-0.22±0.10	-0.52±0.10
N	11	6	6
2.7 (iii)	0.29±0.04	-0.13±0.03	-0.42±0.03
N	10	8	8

Notes: (*) % spacing change relative to mean value at rest,'positive and negative means a spacing increase and decrease, respectively; (**) % spacing change relative to mean value at P_O, negative value implies a spacing decrease; (i) Excess % spacing change of ca. 5.1 nm layer line above that of the 5.9 nm layer line. Positive and negative values mean increase and decrease, respectively; (ii) Values deduced from the spacing changes in the ca. 5.9 and 5.1 nm actin layer lines, and; (iii) Values deduced from direct measurement of the spacing changes of the ca. 2.7 nm actin meridional reflection.

Table 3

Spacings (in nm) of the 3M and 6M	REST	PLATEAU	RELEASE
3M	14.34±0.008	14.430±0.023	14.336±0.011
		14.645±0.012	
6M	7.118±0.004	7.239±0.008	7.119±0.005
	7.179±0.004	7.293±0.006	7.185±0.006
N	10	11	6