Variations of the Contractile Apparatus in Smooth and Striated Muscles

X-ray diffraction studies at rest and in contraction

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ABSTRACT Structural information is presented for three muscle systems—mammalian smooth muscle at rest and partially active, living toad striated muscle at rest and contracting, and glycerinated rabbit psoas muscle under various conditions of pH and ionic environment. In the smooth muscle no evidence of organized myosin filaments has been found. In the striated muscle the myosin-to-actin distance can vary widely, according to sarcomere length and to muscle treatment, both at rest and during contraction. In the discussion it is suggested that muscle should be considered as a colloidal system and that there need not necessarily be any chemical bonding (cross-linking) involved in the contractile process.

It seems very likely that the basic contractile event is the same in all types of muscle, and possibly in other motile systems as well, and it is therefore strange that there is such diversity of muscle structure in the animal kingdom. At King's College J. Hanson, J. Lowy, B. M. Millman,[1] C. R. Worthington,[2] and I, over a period of 10 years, have been studying the structure and function of many very diverse types of muscle, hoping that the structural similarities which we observed would give us clues to the nature of the fundamental contractile process in muscle. We have used the complementary structural techniques of electron microscopy and X-ray diffraction and have in several cases also investigated the physiological properties of the muscles which we studied.

The results of the structural researches (together with those of other laboratories) have been reviewed in recent articles (1, 2), and I shall not repeat the full bibliography here. It has become apparent that, at least on the level at which we work, there are very few structural generalizations which apply

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to all the muscles which have been studied. The position can be summarized as follows: In all types of muscle studied there are two types of filament (but see below), thin filaments containing actin and showing a characteristic structure which is very similar in different types of muscle (3), and thick filaments which contain myosin but have very diverse sizes and structure in different types of muscle. In many instances these thick filaments have been shown to bear projections ("cross-bridges"), and in certain cases the projections have been shown to be the globular heads of the myosin molecule, which bear the ATPase site. In all cases the X-ray diffraction studies have shown that the structure of these thick filaments involves a period which is an integral multiple of 144 A (4). For vertebrate myosin it is 3 × 144 A (5, 6), for paramyosin it is 5 × 144 A (7), and for insect myosin no information concerning the multiplier has yet been published (8, 9), though Reedy has X-ray and electron microscope results which suggest that the period is 1140 A, within the experimental error 9 × 144 A.[3]

Any period determined by X-rays alone may subsequently be shown to be only a submultiple of the true period, since all the indices given to the reflections can be multiplied by any given integer, and there may be systematic absences of some reflections. This comment applies to the periods given above for vertebrate myosin, and for paramyosin, and also to some recent diagrams which Millman and I have obtained from the striated adductor muscle of the clam *Pecten maximus*. Here the period in the thick filaments is 2 × 144 A, or an integral multiple of this period. Hanson and I have made preliminary X-ray investigations of the body wall muscle from the annelid *Myxicola*, where the thick-filament period is based on 144 A but the multiple seems different again, although we cannot yet be certain of this.

MAMMALIAN SMOOTH MUSCLE

The apparent exception to the "two types of filament" rule is the smooth muscle of mammals (similar muscles may possibly be found in other phyla also). In this muscle, which has been studied extensively in the electron microscope, there is one clear set of filaments, which, from their size and their appearance in homogenized preparations, may reasonably be supposed to be actin filaments. There have also been various reports of a second set of thicker filaments, but the status of these filaments remains in considerable doubt. The electron microscope and biochemical evidence on vertebrate smooth muscles is summarized, with a full bibliography, in a recent paper by Shoenberg, Rüegg, Needham, Schirmer, and Nemetchek-Gansler (10). Shoenberg et al. conclude that "in relaxed vertebrate smooth muscle the

[3] Personal communication from M. K. Reedy of the Department of Physiology, University of California at Los Angeles (formerly of the Laboratory of Molecular Biology, Medical Research Council, Cambridge University).

contractile protein consists of actin in filament form and disaggregated myosin, or myosin aggregated to such a small degree that the aggregates are not visible by negative staining."

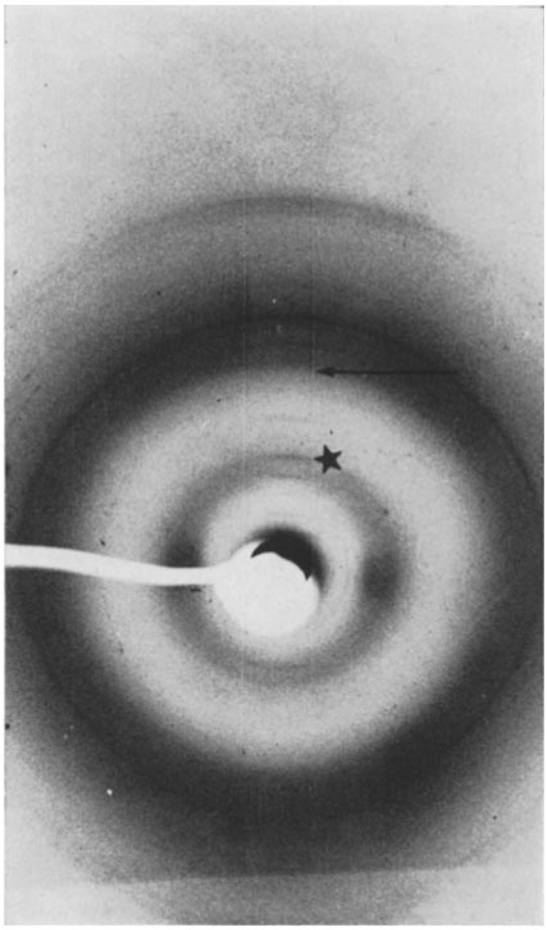

FIGURE 1. High-angle X-ray pattern from a dried specimen of the taenia coli muscle of the guinea pig, tilted to the correct angle for the 5.1 A α-reflection. The arrow marks the region in which this reflection should appear, and for calibration purposes the 9.14 A meridional actin reflection is marked with a star. The continuous rings probably arise from the Lotmar-Picken substance, taurine (see reference 6). Taken by Dr. Arthur Elliott on his toroidal focusing camera.

Some years ago I studied the X-ray diagram given by a particular mammalian smooth muscle (the taenia coli muscle of the guinea pig) and could not find, either at high angles or at low angles, any evidence that myosin was present in filamentous form, though a clear and well-aligned actin pattern could be seen (6). Huxley[4] has confirmed these observations but re-

[4] H. E. Huxley. Personal communication cited by Shoenberg et al. (10).

marked that this was not conclusive evidence since the myosin pattern was very easily disturbed. Now it is true that the myosin low-angle pattern is in general more difficult to observe than is the actin pattern, and that its visibility depends on preparative treatment, but it remains unique that under none of the conditions so far examined (dried muscle; living muscle without stimulation; glycerol-extracted muscle; muscle fixed in formaldehyde, glutaraldehyde, and osmium, with or without phosphotungstic acid staining) have there been any low-angle reflections from myosin or its homologues in

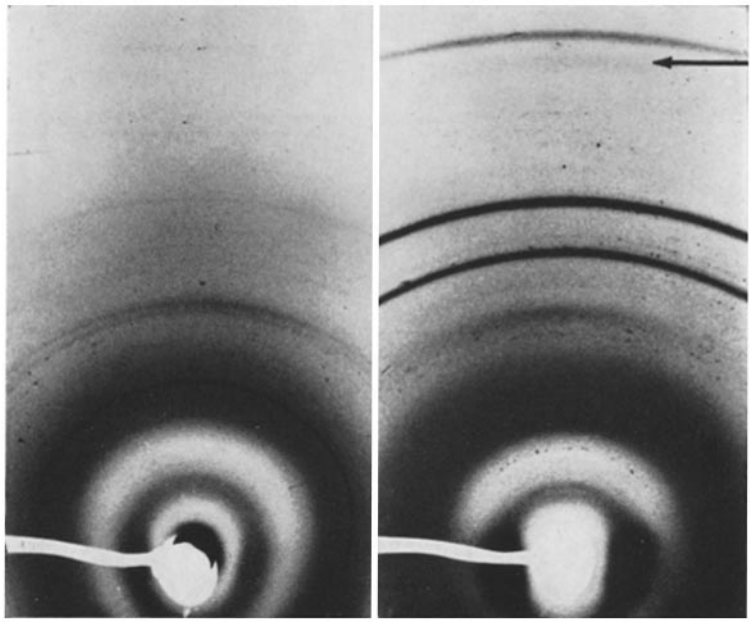

FIGURE 2. High-angle X-ray patterns from dried muscle specimens taken at the same specimen to film distance, and tilted to the correct angle for the 1.5 A α-reflection. Left, taenia coli, 1.5 A reflection absent; right, ABRM, 1.5 A reflection marked with an arrow. Taken by Dr. Arthur Elliott on his toroidal focusing camera.

this muscle. B. M. Millman and I have taken further X-ray patterns of this muscle without finding any new reflections. We have, however, succeeded by chance in obtaining low-angle diagrams from the muscle during partial activity. When the muscle is lightly loaded, and hung in an atmosphere of oxygen with a Ringer drip, it goes into a state of rhythmic contraction and relaxation, stimulating peristalsis. X-ray patterns taken in this state again show no myosin low-angle reflections. We have not yet used the stroboscopic method which we developed for X-ray studies of contracting vertebrate striated muscle (11), but the muscle was at least contracting for a sizable fraction of the total exposure without low-angle myosin reflections becoming apparent.

We have also carried out further studies on the high-angle region of the X-ray diagram. Fig. 1 is the high-angle pattern of a dried specimen of taenia coli tilted to the appropriate angle for the 5.1 A α-reflection, which is not, however, observed. Nor does the equatorial diffraction appear to be that of an α-protein (see reference 6). Fig. 2 is a comparison between dried specimens of taenia coli and of the anterior byssus retractor muscle of *Mytilus edulis* (ABRM) tilted to the appropriate angle for the 1.5 A α-meridional reflection. This reflection is clearly seen from the ABRM (arrow), but not from the taenia coli. Now since the wide-angle α-pattern is so ubiquitous in fiber diagrams, it seems legitimate to conclude that its absence indicates the absence of oriented α-protein, at least in any sizable fraction. It is possible, though hardly likely in this muscle alone, that oriented myosin filaments may have been dissolved during the drying process by local variations in salt concentration. Otherwise it seems difficult to escape from the conclusion of Shoenberg et al. (10) that "in relaxed vertebrate smooth muscle the myosin exists in a colloidally-dispersed phase."

THE FILAMENT LATTICE OF CONTRACTING VERTEBRATE STRIATED MUSCLE

As was pointed out in a paper read in 1963 to a previous discussion meeting on muscular contraction (6), the X-ray results of Elliott, Lowy, and Worthington (12) on living, relaxed frog striated muscle show that, over the range in which contraction is possible (sarcomere lengths, 2.0–3.5 μ), the surface-to-surface distance between actin and myosin filaments changes from 110 A to 40 A, making reasonable assumptions about the diameters of the actin and myosin filaments. At that time Lowy, Millman, and I set out to show how this distance changed with sarcomere length in contracting muscle, or whether, indeed, it was constant at all sarcomere lengths, as might be expected on the simplest interpretation of "cross-bridge linking" theories of contraction. The stroboscopic method which we developed, and the results of our experiments, have been published (11, 13), and in this paper I wish simply to draw from the results a few personal conclusions concerning the contractile process. Fig. 3 shows the behavior of the equatorial (1,0) spacing, $d_{1,0}$, in resting and contracting toad sartorius muscle (we used toad muscle because of the longer twitch duration). It may be observed that the "constant-volume" behavior of resting muscle (see reference 12) is repeated in contracting muscle, indeed over a wider range, since it is much easier to hold contracting muscle at short sarcomere lengths than it is to hold resting muscle, which tends to pull out under its own weight (see Fig. 3). Making the same assumptions about filament diameters as before, the surface-to-surface actin-to-myosin distance varies between 50 A (at 3.5 μ sarcomere length) and 130 A (at 1.8 μ) in toad muscle contracting actively. (In resting toad muscle this parameter varies

from 50 A at 3.6 μ to 110 A at 2.0 μ.) This is a striking variance in active muscle, and I feel that it demands attention, and explanation, from advocates of "cross-bridge linking" theories (see discussion below).

Millman and I, studying the meridional diffraction from ABRM during tonic contraction (Fig. 4 and Table I), were able to show that there was no over-all change in the arrangement of the molecules within either the actin

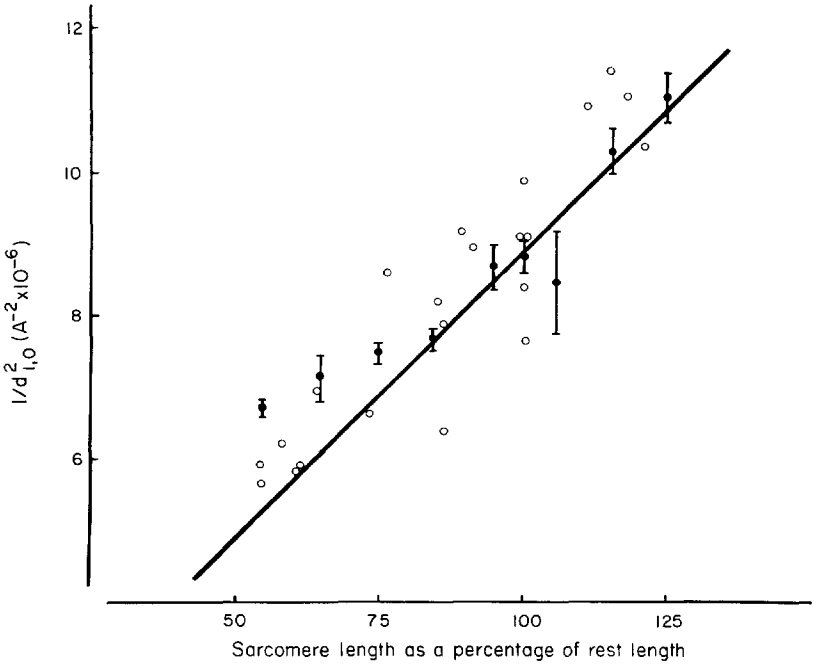

FIGURE 3. The square of the reciprocal of $d_{1,0}$ plotted against muscle length (rest length is a sarcomere spacing of 2.9 μ) for resting toad sartorius muscle (●, averaged from many observations with standard deviation shown) and for contracting muscle (○, each one a single experiment). The line is the best least squares fit to the solid points, omitting the three lowest. *Figure reprinted by permission from The Journal of Molecular Biology, 1967, 25:13.*

or paramyosin filaments, and these studies provide strong evidence against the hypothesis that tonic contraction in such muscles is caused by a change of state (crystallization) in the paramyosin component of the muscle (14). Lowy, Millman, and I went on to show from meridional X-ray patterns that in toad striated muscle also there is no over-all change in the arrangement of the molecules in either the actin or the myosin filaments (Table II), and we were able to confirm the observation of Huxley, Brown, and Holmes (15) that the 432 A myosin layer lines become much less intense (relative to the meridional reflections) in contracting as compared with resting muscle. [Huxley et al. (15), at about the same time as ourselves, had also shown the constancy of the

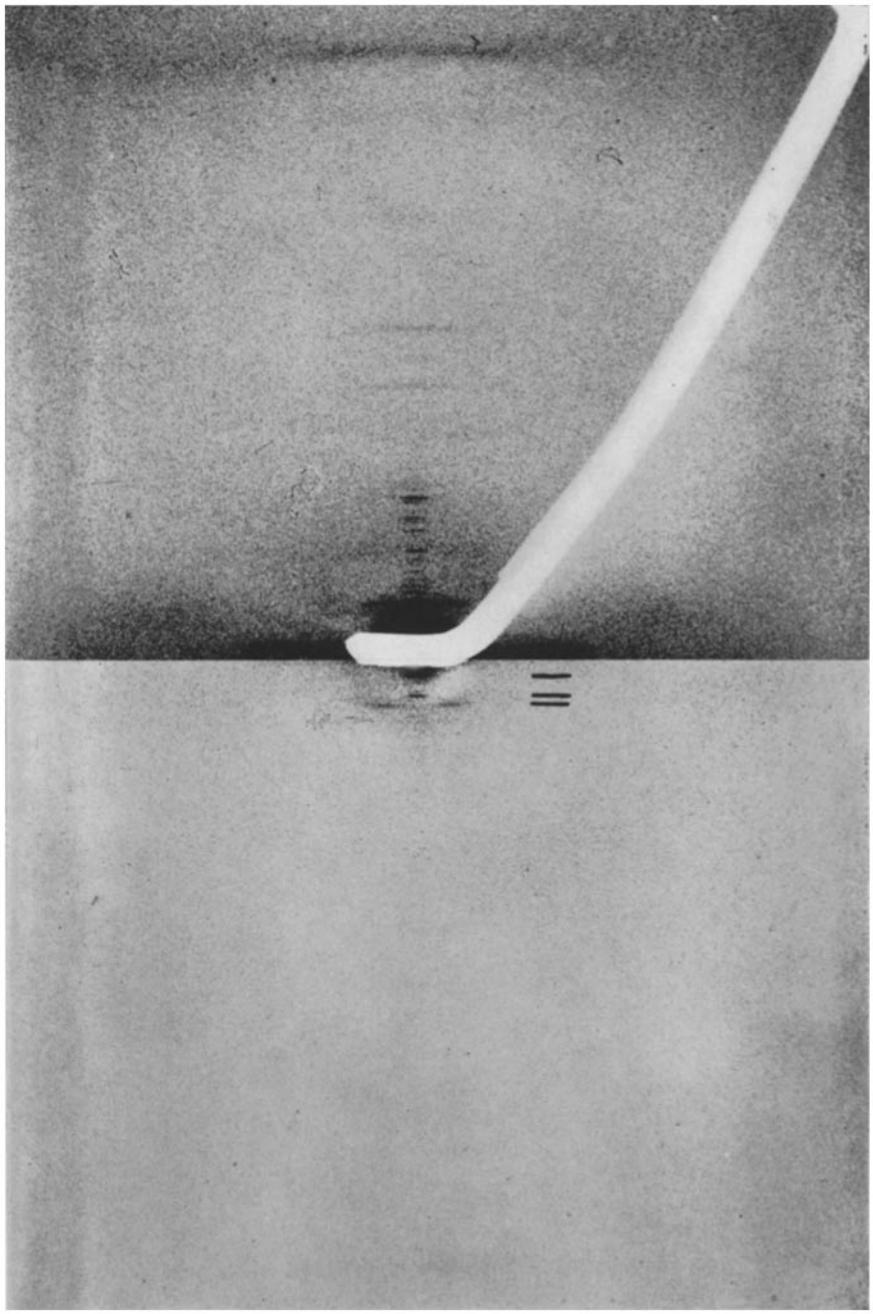

FIGURE 4. X-ray diffraction pattern from contracting ABRM. The figure was made using two films from the same X-ray exposure, where one film was directly on top of the other. The upper half of the figure is from the top film; the lower half is from the second. The actin (58.2 A, off-meridional) and paramyosin (71.6 and 141 A, meridional) reflections which were measured (Table I) are marked by lines. *Figure reprinted by permission from Nature, 1965, 206:824.*

actin and myosin axial spacings in frog striated muscle during contraction.] The myosin layer lines had previously been observed by Worthington (8) in insect muscle and by myself (6) in living frog muscle, and had been ascribed (6) to the projections on the myosin filaments which Huxley's very beautiful electron micrographs had long before demonstrated (16). The implications of

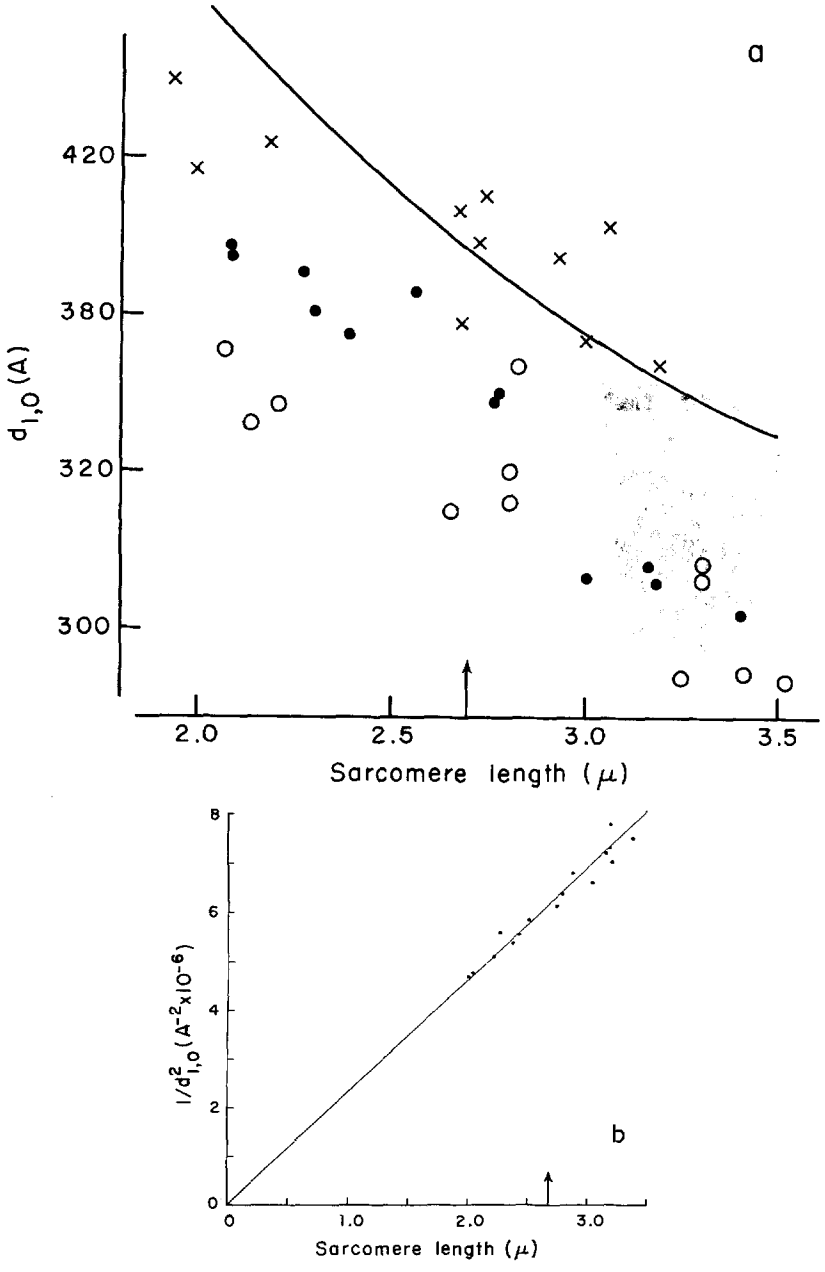

FIGURE 5

these results will be discussed at the end of this paper, but first some other relevant work will be described.

THE FILAMENT LATTICE OF GLYCERINATED MUSCLE

A recent and welcome recruit to our team, Elizabeth Rome, has been investigating the behavior, with sarcomere length, of the equatorial X-ray diffraction pattern from the classical glycerinated muscle, rabbit psoas muscle, where there had been some differences in the published observations. Rome has used a helium-neon gas laser as a light source to make accurate light diffraction measurements of sarcomere length (s) and, has been able to confirm (17) that the relative intensities of the equatorial reflections from glycerinated muscle vary with sarcomere length in the same way as do those from living muscle (12). She has also discovered a significant difference in the lattice behavior between glycerinated muscles examined in glycerol (50% glycerol in water–M/150 phosphate buffer) and glycerinated muscles examined in salt solution (0.1 M KCl–1 mM $MgCl_2$–M/150 phosphate buffer).

Fig. 5 shows the behavior in glycerol. The solid line in Fig. 5*a* is for glycerol at pH 7. Individual points are not shown for this pH, but in Fig. 5*b* the individual points at pH 7 are plotted in the same manner as in Fig. 3, to show the clear constant-volume effect, precisely that of living muscle (cf. Fig 3, but note that the values of d^2s differ for toad and rabbit muscle). The other points in Fig. 5*a* are individual measurements at other pH's, 6.3, 6.6, and 7.9 (see the caption). The trends of these points are similar at the various pH's, but at the pH's below neutral $d_{1,0}$ decreases markedly with pH, from 395 A at pH 7 to about 320 A at pH 6.3, both at rest length (s = 2.7 μ).

Fig. 6 shows the behavior when the glycerinated muscle is transferred to the salt solution. In this case the dependence of $d_{1,0}$ upon s is strikingly different. Instead of a constant volume relationship (d^2s = constant), Rome's results show a linear dependence of $d_{1,0}$ upon s, of the form, at pH 7, $d_{1,0}$ = 475 − 31.7 s ($d_{1,0}$ in Ångströms, s in microns). At pH 6.3 and 7.7, the trends were similar, but the values of $d_{1,0}$ at a particular s were smaller at pH 6.3, and

FIGURE 5. *a*. Lattice spacing, $d_{1,0}$, plotted against sarcomere length for specimens of rabbit psoas muscle extracted and examined in glycerol solution at pH 6.3, 6.6, and 7.9. The points ○, ●, and × represent individual results at pH 6.3, 6.6, and 7.9, respectively. The curve represents the case for specimens extracted and examined in glycerol solution at pH 7.0, and is calculated from the constant volume equation, $(d_{1,0})^2s$ = [4.32 ± 0.15] 10^9 A^3, derived from Fig. 5*b*. The "rest length" (2.7 μ) is indicated by the arrow.

b. The square of the reciprocal of $d_{1,0}$ plotted against sarcomere length for specimens of psoas extracted and examined in glycerol solution at pH 7.0. Each point represents an individual result, and the best straight line through the origin has been drawn. *Figure reprinted by permission from The Journal of Molecular Biology. In press (17).*

TABLE I

ACTIN AND PARAMYOSIN SPACINGS FROM RESTING AND CONTRACTING ABRM

Spacing	Resting muscle	Contracting muscle
	A	*A*
Group A		
Actin	58.8±0.13* (8)‡	58.6±0.17 (8)
Paramyosin	72.0±0.18 (8)	72.0±0.08 (8)
	143±0.6 (5)	143±0.6 (7)
Group B		
Actin	58.6±0.25 (17)	58.4±0.16 (13)
Paramyosin	72.3±0.16 (17)	72.0±0.20 (13)
	143±0.5 (12)	144±1.1 (9)

Group A includes only muscles from which both a resting and a contracting pattern were obtained. Group B includes all muscles from which patterns were obtained (including those in A). Data taken from Millman and Elliott (14).

* Standard deviation from the mean.

‡ Number of muscles. Note that in some patterns the "144 A" paramyosin reflection could not be measured.

TABLE II

ACTIN AND MYOSIN REFLECTIONS FROM RESTING AND CONTRACTING TOAD SARTORIUS MUSCLE

Reflection order	Resting muscle	Contracting muscle
	A	*A*
Actin		
7	59.5±0.12* (32)‡	59.3±0.22 (10)
1	404±3.3 (23)	407±11 (3)
Myosin		
11	30.3±0.06 (9)	—
10	43.5±0.17 (7)	44.0 (1)
9	49.7±0.87 (4)	48.8 (1)
8	54.1±0.17 (6)	—
7	60.1±0.09 (3)	—
6	71.8±0.20 (27)	72.2±0.31 (9)
5	86.2±0.34 (17)	86.8±0.69 (4)
4	107.5±0.47 (17)	107.8±1.14 (6)
3	143.7±0.39 (28)	143.5±0.79 (11)
2	217±1.5 (19)	218±3.7 (7)
Avg basic myosin periodicity	432.4±0.65 (137)	432.8±1.43 (39)

Data taken from Elliott et al. (13).

* Standard deviation from the mean.

‡ Number of measurements.

greater at pH 7.7, than they were at pH 7 and the same s. The difference between $d_{1,0}$ under constant-volume conditions (in glycerol) and linear-dependence conditions (in salt solution) is greatest at the shortest sarcomere lengths.

Rome was able to show that the type of relationship, and the actual value of $d_{1,0}$, did not depend on the history of the muscle specimen, but only on the final solution in which it was examined. Her results are surprising in that, as far as the lattice is concerned, the fluid of the contractile apparatus in living

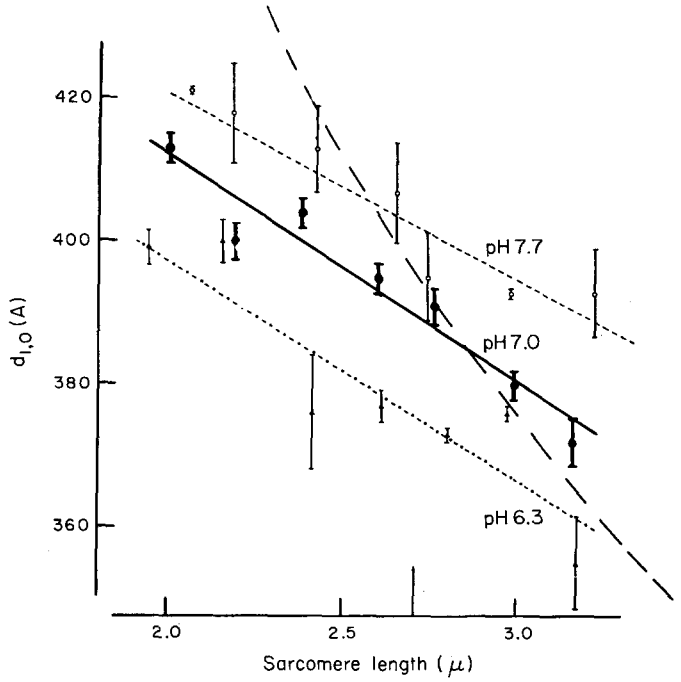

FIGURE 6. Lattice spacing, $d_{1,0}$, plotted against sarcomere length for specimens of rabbit psoas muscle extracted with glycerol at pH 7.0 and examined in salt solution. The points ▲, ●, and ○ on the three curves represent values of $d_{1,0}$ averaged over 0.2 μ sarcomere length units obtained from specimens examined in the salt solutions of pH 6.3, 7.0, and 7.7, respectively. Each vertical line represents the standard error of the mean at that point, and the best straight line has been drawn for each set of points. The large dashed curve represents the case for specimens extracted and examined in glycerol solution at pH 7.0, and is calculated from the constant volume equation derived from Fig. 5*b*. The "rest length" (2.7 μ) is indicated by the arrow. *Figure reprinted by permission from The Journal of Molecular Biology. In press (17).*

muscle can be replaced in glycerol-extracted muscle by an ionically inert medium (glycerol), but not an ionic medium (salt solution). This suggests that in the localities in living muscle where the interfilament forces are acting, either there are very few free ions, or if there are a considerable number present, they must be of a specific nature (distinct from the salt solution) such that they have no effect on the forces determining the dimension of the filament lattice. Rome has also pointed out that the pH dependence of the lattice

dimensions indicates that there are forces of an electrostatic nature affecting the lattice dimensions of glycerol-extracted muscle.

DISCUSSION

If indeed mammalian smooth muscles have no myosin filaments, then logically only organized actin filaments appear necessary for contraction. Since the experimental evidence is not yet beyond doubt, however, it seems better to treat such a radical idea with caution at the present time, and to concentrate on the systems where there is no doubt that two types of filament exist.

Our results, which show that there is no over-all change in the arrangement of the molecules in either the actin or the myosin-containing filaments in ABRM (14) and toad sartorius muscle (11, 13) during contraction [see also Huxley et al. (15)], leave no doubt about the essential truth of the sliding filament model for length changes in living muscle (18, 19) and underline the conclusions of our earlier study of the equatorial X-ray pattern of living, relaxed frog sartorius muscle (12), where we showed that the equatorial intensity variation with length could be simply explained in terms of the variable overlap between the two sets of filaments.

A very significant aspect of our results from contracting muscle is the light which is thrown on the nature of the interaction between the actin and myosin filaments. Since the contractile process can take place when the average surface-to-surface distance between actin and myosin is 50 A, but can still take place when it is 130 A, it is difficult to maintain "chemical cross-bridge linking" theories of contraction unless ad hoc assumptions are made. These assumptions can be of three types: the filaments may swell to maintain contact; the "cross-bridges" may be folded, or bent, at long sarcomere lengths, or may extend by 80 A (160%); or the interaction may take place only at places where Brownian motion gives chance contact. There is little or no experimental evidence for filament swelling; neither is there any evidence from X-ray diffraction or electron microscopy of cross-bridge folding at long sarcomere lengths, and a 160% extension seems energetically unlikely. Brownian contact would seem a rather inefficient mechanism. For these reasons I have preferred to discard such ad hoc assumptions and to regard muscle as a colloidal system, where the balance of electric double-layer effects, van der Waals' attraction, and hydration effects (20) controls the interfilament spacing in the lattice, both in resting and in contracting muscle, and where there is a clear analogy with the classical work of Bernal and Fankuchen on colloidal systems of tobacco mosaic virus (21). The results and conclusions of Rome's work (17) seem to fit in with this picture and also suggest that in the local environment of the filaments the ionic strength may not be as high as has hitherto been supposed. The short range (10 A) of electrical double-layer effects in 0.1 M KCl has always been a difficulty in considering muscle as a

colloidal system. Worthington has pointed out (22) that the maximum tension in muscle is 5×10^{-6} dyne (between sites, assuming that the myosin projections are the active sites) and that this is 40 times less than the force (about 2×10^{-4} dyne) required to rupture a weak interaction of 1 kcal $mole^{-1}$ (which is the order of magnitude of a hydrogen bond). This suggests that the forces involved in the maintenance of the lattice, and the contractile process, are weaker than those of chemical bonds, and the type of forces involved in colloidal systems could well fit the facts.

Now, the considerations which I have set out should not be taken to mean that I do not believe that the projections (cross-bridges) are involved in maintaining the regular lattice in resting muscle, and/or in the contractile process. On the contrary, I think it very likely that the projections are the sites of charge concentration which give rise to the double-layer effect. The X-ray and electron microscope work of Reedy, Holmes, and Tregear (23) on insect muscle has demonstrated a clear change of orientation of the myosin projections between glycerinated muscle in rigor or in relaxing solution, and the X-ray work of Huxley's group on contracting frog muscle (15), confirmed by our work on contracting toad muscle (12, 13), shows that the projections become disordered when the muscle contracts. These facts are significant, and require explanation, though it should be borne in mind that any contractile activity will probably produce waste metabolic heat, and consequently increase the Brownian motion of the projections, whose X-ray pattern is in any case difficult to observe compared to the backbone actin and myosin reflections (6).

Concerning the contractile process, I feel that the basic question is whether the changes observed in the myosin projections are the physical cause of a contractile process which involves linking and breaking of cross-bridges, or are the effect of a mechanism in which physical contact between the actin and myosin filaments is not necessary and chemical bonds are not involved.

I am indebted to Professor Sir John Randall for the provision of facilities, and to Professor Jean Hanson, Dr. Jack Lowy, Dr. Barry Millman, and Dr. Roy Worthington for constant and stimulating discussion over many years, though I need hardly add that none of these colleagues would necessarily agree with the discussion paragraphs of this paper. I wish to thank Miss Elizabeth Rome for allowing me to use the unpublished results of her work, and to acknowledge that many of my recent thoughts on the contractile process have arisen from her work, and from discussions with her. I also wish to thank Dr. Arthur Elliott, who used his toroidal focusing camera to take the X-ray diagrams of Figs. 1 and 2.

REFERENCES

1. HANSON, J., and J. LOWY. 1965. *Brit. Med. Bull.* **21**:264.
2. ELLIOTT, G. F. *In* Contractile Systems, E. Anderson-Cedergren, editor. North Holland Publishing Company, Amsterdam. In press.
3. HANSON, J., and J. LOWY. 1963. *J. Mol. Biol.* **6**:46.

4. ELLIOTT, G. F., and C. R. WORTHINGTON. 1958. *J. Physiol.*, (*London*). **149**:32P.
5. WORTHINGTON, C. R. 1959. *J. Mol. Biol.* **1**:398.
6. ELLIOTT, G. F. 1964. *Proc. Roy. Soc.* (*London*), *Ser. B.* **160**:467.
7. BEAR, R. S. 1944. *J. Am. Chem. Soc.* **66**:2043.
8. WORTHINGTON, C. R. 1961. *J. Mol. Biol.* **3**:618.
9. ELLIOTT, G. F. 1965. *J. Mol. Biol.* **13**:956.
10. SHOENBERG, C. F., J. C. RÜEGG, D. M. NEEDHAM, R. H. SCHIRMER, and H. NEMETCHEK-GANSLER. 1966. *Biochem. Z.* **345**:255.
11. ELLIOTT,G. F., J. LOWY, and B. M. MILLMAN. 1965. *Nature.* **206**:1357.
12. ELLIOTT, G. F., J. LOWY, and C. R. WORTHINGTON. 1963. *J. Mol. Biol.* **6**:295.
13. ELLIOTT, G. F., J. LOWY, and B. M. MILLMAN. 1967. *J. Mol. Biol.* **25**:31.
14. MILLMAN, B. M., and G. F. ELLIOTT. 1965. *Nature.* **206**:824.
15. HUXLEY, H. E., W. BROWN, and K. C. HOLMES. 1965. *Nature.* **206**:1358.
16. HUXLEY, H. E. 1957. *J. Biophys. Biochem. Cytol.* **3**:631.
17. ROME, E. 1967. *J. Mol. Biol.* In press.
18. HUXLEY, A. F., and R. NIEDERGERKE. 1954. *Nature.* **173**:971.
19. HUXLEY, H. E., and J. HANSON. 1954. *Nature*, **173**:973.
20. VERWEY, E. J. W., and J. TH. G. OVERBEEK. 1948. Theory of the Stability of Lyophobic Colloids. Elsevier Publishing Company, Amsterdam.
21. BERNAL, J. D., and I. FANKUCHEN. 1941. *J. Gen. Physiol.* **25**:111.
22. WORTHINGTON, C. R. 1964. *In* Biochemistry of Muscle Contraction. J. Gergely, editor. Little, Brown and Company, Boston. 511.
23. REEDY, M., K. HOLMES, and R. TREGEAR. 1965. *Nature.* **207**:1276.

Discussion

Dr. Philip W. Brandt: We have exactly the same data as Dr. Elliott, but from electron microscopy. The lattice volume is a constant volume and after preparation for electron microscopy it is exactly the same volume as the in vivo volume. This is undoubtedly due to "hydration" of the lattice by the Epon. It seems clear to us that a number of the electron microscopical studies that have been done on myofilament length and the appearance of cross-bridges depended on fixing the muscle in conditions which depolarize it prior to the actual chemical setting of the structure. This allows a variety of things to happen; mostly the lattice tends to collapse. And in the collapsed lattice a lot of things, such as cross-bridges and constant myofilament lengths, are much more visible than they appear to be at all sarcomere lengths in the maintained lattice.