THE STRUCTURE OF ACTIN-RICH FILAMENTS OF MUSCLES ACCORDING TO X-RAY DIFFRACTION*

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INTRODUCTION

In the accompanying paper[1] we have described the structure of paramyosin in molluscan muscles. A second type of fibrous muscle component is possibly of greater significance, since it has been found in all muscles thus far examined, in smooth and striated varieties from vertebrate and invertebrate animals (Schmitt, Bear, Hall, and Jakus, 1947).

When first discovered by means of x-ray diffraction this second component was designated type II (Bear, 1945). Subsequent x-ray examination of actin films by Astbury and Spark (1947; see also Astbury, 1947, 1949) indicated that it is an actin-rich fibrous system, if not composed of actin alone. In what follows for brevity we shall call this fibrous system actin, although very complete knowledge of the diffraction properties of isolated actin has not been available during most of this investigation, which deals only with results obtained from intact dried muscles.

In the original diffraction work unambiguous index assignments could not be made for the observed reflections. It was clear, however, that the axial identity period was in the range 350 to 420 A. Subsequent electron-optical investigations of striated muscle have revealed a similar axial periodicity (*ca.* 400 A, *cf.* Draper and Hodge, 1949), which has come to be considered, though without detailed justification, as related to the same system observed by the early diffraction studies. Recently Philpott and Szent-Györgyi (1954)

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[1] Throughout the present paper considerable reference is made to the discussion concerning paramyosin (Bear and Selby, 1956), without specific citation. There background was developed for interpretation of diffraction by nets or helices and for general terminology useful in description of macromolecular systems of this kind. While the colloidal elements of paramyosin fibers are termed *fibrils*, after usage now common with many fibrous structures, in the present case the thinner actin-rich elements are called *filaments*, since in many muscles these are subdivisions of the relatively gross myofibrils.

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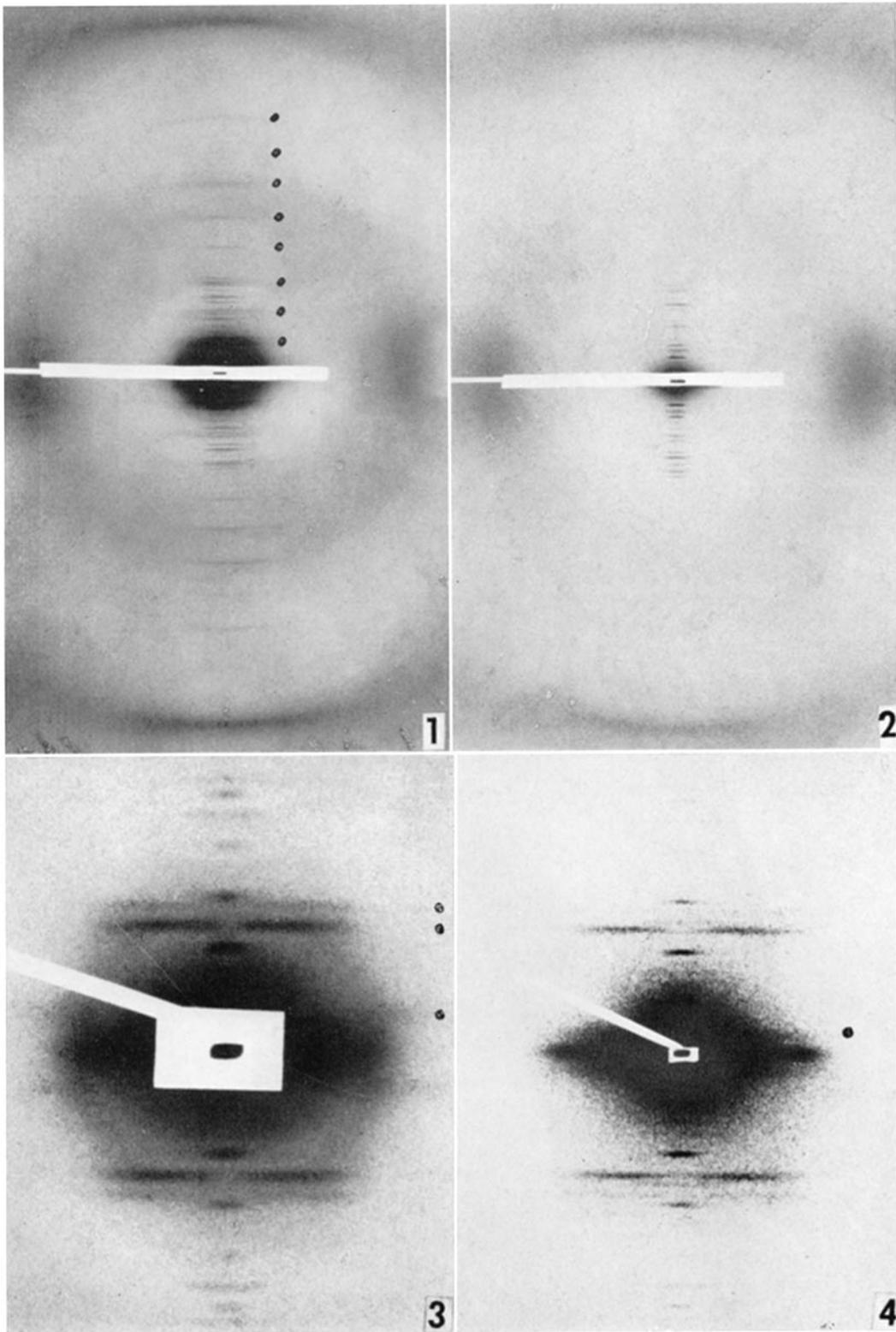
have shown electron-optically that the light meromyosin portion of myosin is also characterized by an axial periodicity of about 400 A.

The largest spacing directly observed in the early diffraction work on muscle was 58 A, apparently the sixth or seventh order of the axial identity period. Since then improved techniques have permitted Cannan (1950) and Huxley (1953 a) to examine reflections corresponding to spacings as large as 400 to 420 A. This paper presents in detail the results of Cannan, particularly in the light of experience gained from study of the more distinct, but in some respects similar, diffraction by paramyosin.

Experimental Methods

The diffraction procedures, as well as the sources and methods of handling the molluscan muscle strips, were essentially the same as the corresponding ones employed in the paramyosin investigation. The chief exception was that, instead of the "white" part, the "tinted" portion of the adductor muscle of *Venus mercenaria* was used. This latter source is more rich in the actin component. While other muscles, such as invertebrate ones, exhibit the actin component in diffraction without contamination with paramyosin, there is some advantage to tolerating the paramyosin in molluscan samples because it confers some degree of stability on the specimens.

In addition to pinhole cameras capable of resolving 400 and 800 A, the 600 A slit-collimated camera of Bolduan and Bear (1949) was employed in examination of the "moderate-angle" reflections (see below). This camera permitted more rapid and intense registration of the high-order, near-meridional reflections of the 406 A axial period, with adequate resolution maintained meridionally. Even some distinction of relative transverse locations of the reflections was possible.

RESULTS

Description of Diffraction Patterns

As can be seen from the wide-angle reflections of Figs. 1 and 2, the tinted *Venus* muscle, like the white portion, exhibits the α-type diagram of the KMEF class of fibrous proteins. However, much of this, if not all, derives

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FIG. 1. Wide- to moderate-angle x-ray diffraction pattern of dry, tinted *Venus* adductor muscle. The relatively large diffuse diffractions, chiefly the intense arcs on the equator (horizontal midline) and meridian (vertical midline), are typical of α-type fibrous proteins. The longer lines near the meridian and marked with dots arise from the actin-rich filaments under discussion in the text. The shorter central meridional lines are due to the paramyosin component of the muscle.

FIG. 2. Diffraction by a preparation similar to that of Fig. 1 but treated with 10 per cent ethanol at room temperature for 3 days before drying. Only the α-type wide-angle diffraction and the paramyosin small-angle diffraction remain.

FIG. 3. Small-angle diffraction by dry, tinted *Venus* adductor muscle, taken with the 400 A camera. Dots mark levels of important reflections corresponding to k indices 2, 7, and 8, reading upward. Other scattering is like that exhibited by white *Venus* muscle (paramyosin).

FIG. 4. Same as Fig. 3, except taken with a camera having better angular resolution (the 800 A camera). The dot marks the level of the $k = 1$ reflection just emerging from central diffuse scatter.

from the paramyosin. In all muscles the α-type diagram is observed, but where paramyosin is lacking the myosin component, also an α-type fibrous protein, is still present. Actin constitutes generally a minor proportion of the protein of any muscle (*ca.* 15 per cent in vertebrate examples), so that studies on whole muscle do not easily reveal the small-scale structure of actin.

Astbury and Spark (1947) found that α-type diffraction was not exhibited by their actin specimens. While intimating some similarities to members of the β-class of fibrous proteins (*e.g.* feather), from diffraction comparisons at small angles, these authors did not clearly classify actin at wide angles. The small-scale structure of actin must be considered as not yet apparent from diffraction studies.

In considering the large-spacing reflections, it is convenient to distinguish two diffraction areas as follows: (*a*) moderate-angle diffraction, represented by the central portions of Figs. 1 and 2, and comprising spacings from 7 to 60 A; and (*b*) true small-angle diffraction typified by Figs. 3 and 4, showing the reflections which are given k indices 1, 2, 7, and 8 below. The moderate-angle diagrams were obtained with slit collimation, but the small-angle ones were from pinhole cameras and show close to true diffraction shapes.

The actin reflections are sharp in the meridional direction, indicating that the diffraction is by long thin filaments whose structural periodicities repeat many times along their axes. In the transverse direction, however, reflections are streaked out considerably along layer lines, even on pinhole photographs, so much so that it is difficult to specify satisfactorily the transverse rotation-diagram coordinates (ξ). The moderate-angle streaks extend considerably on either side of the meridian, and they are also grouped along the meridian, in such a way as to give the general impression (at poor angular resolution) of long lines occurring as successive orders of an axial identity period of about 55 A.

Closer examination, particularly at small angles, shows, however, that the layered reflections have further structure indicating the presence of a larger axial identity period. Table I describes all observed reflections and indexes them (k) according to an identity period (b) of somewhat over 400 A. Those at $k = 1$ and 2 may not belong to the same system as the others, however, and are further discussed as "non-net reflections" during the following analysis. Another equally satisfactory indexing of the higher layer lines is presented in the k' column. Averages of kd or $k'd$ for all layer lines except the first two yield for the possible axial periodicities $b = 406 \pm 6$ A or $b' = 351 \pm 6$ A.

Transverse coordinates (ξ) could be satisfactorily estimated only for the reflections at $k = 2, 7, 8$, which were 0.034, 0.015, and 0.023 radian, respectively. The important reflection at $k = 1$ is too close to the central diffuse scatter, and others at larger k are too badly streaked transversely. The reflections on higher layer lines are extensive in length (a) because of great natural

length, (b) because of inherent rotation of the filaments of the massive fibrous specimens about otherwise parallel axes, to repeat reflections on both sides of the meridian, and (c) also possibly because the reflections belong to row lines which are not orthogonal to the pattern equator (see further below). However, some indication of relative transverse coordinates is provided by the pro-

TABLE I

X-Ray Reflections Exhibited by Tinted Venus Muscle

k	k'	d	h	I	F-actin films	
					d	I
		A			A	
1		400.	(±1)	s		
2		195.	(±2)	m		
7	6	59.	+1	vs		
8	7	51.	−1	s	54 ?	
14	12	28.2	+2	w		
15	13	26.9	0	w	26.8	s
21	18	19.0	+3	w		
22	19	18.8	+1	m	18.1	m
30	26	13.6	0	m	13.5	m
31	27	12.9	−2	w		
37	32	10.9	+1	m	10.9	s
45	39	9.0	0	m	9.1	s
52	45	7.9	+1	w	7.9	m
60	52	6.8	0	m	6.8	s

The table describes x-ray reflections observed with *Venus* adductor muscle (tinted component), beyond those of paramyosin described elsewhere (Bear and Cannan, 1956). The first five columns deal with the experimental data and the indexing discussed in the text. The last two columns present comparable measurements for F-actin films cited from Astbury (1949). The layer-line indices k and k' show two ways that the reflections can be indexed along the pattern meridian. The axially projected spacings d are derived from the corresponding experimentally measured rotation-diagram coordinates, ζ, according to λ/ζ, in which λ is the wave length of x-rays used (1.54 A). Transverse locations of the diffraction maxima are roughly indicated by the magnitudes of the h indices, whose signs are provisionally adopted in agreement with selection rules discussed in the text. Columns headed I indicate relative intensities in the order of decreasing strength vs, s, m, w.

visionally assigned h indices, which were chosen by pattern examination in the light of the selection rule discussed in the next section.

The diffraction patterns for the tinted muscle also show the three types of relatively diffuse central scatter described for the white portions of *Venus* muscle in the accompanying paper, possibly because of very similar origin. In examining rehydrated samples difficulty was experienced in maintaining satisfactory specimen orientation, with the result that, of the important pair of reflections at $k = 7$ and 8, only an unresolved combination was observed because of arcing.

Sensitivity of Actin Structure to Alcohol

The actin system of reflections exhibits a behavior which is unusual among fibrous proteins. Muscle specimens which had been immersed in 5 to 95 per cent aqueous ethanol before air drying lost the structure yielding the moderate-angle reflections (comparable observations at very small angles were not made). Figs. 1 and 2 compare samples before and after such treatment. Only the wide-angle α-diagram and the paramyosin small-angle reflections survive the alcohol treatment. Similar handling of both fresh and air-dried specimens with other solvents—water, benzene, ether, pyridine, or acetone—did not materially influence the actin diagram (nor the paramyosin reflections). This sensitivity of the actin system to alcohol is a property which should be remembered when attempts are made to examine the actin component, in muscles or isolated, after dehydration or other procedures involving alcohol.

The Actin Net

Standard methods of analysis can be applied only with difficulty to the reflections described above. Analysis would be facilitated by means which would strengthen the reflections relative to background scatter or eliminate interference produced by the accompanying paramyosin reflections. On the other hand orientation of filaments within specimens is good, as shown by the straightness and lack of arcing of layer-line reflections. Indeed, the major sources of the difficulties which prevent simple description of the diffraction are derived from causes inherent in the structure of the actin filaments and the muscle fibers themselves. Thus the wide streaking of the reflections indicates the presence in this system of factors, such as those discussed by Bear and Bolduan (1950), which lead to evidence of apparent natural deficiencies of large-scale order in fibrous systems.

Clues as to the interpretation of the actin pattern were provided by the experience with paramyosin described in the accompanying paper. Detailed examination of the diffraction locations, as expressed in terms of h and k indices, showed a strong preference for expression of reflections satisfying a selection rule similar to that of paramyosin. The actin selection rule is

$$h = \frac{k - 15m}{7},$$

in which m is a test integer running from $-\infty$ to $+\infty$, including zero. Values of m which yield integral h at a given k indicate at which transverse location to expect intensity maxima on that layer line. Inspection of the measurements in Table I shows that all observed reflections occur on layer lines (k) consistent with the selection rule at small h, except for the two central reflections ($k = 1, 2$). The latter appear not to be as far removed from the meridian as

the selection rule would require. They should be at $h = -2, -4$, respectively, instead of near the ± 1 and ± 2 locations observed. These two exceptions are distinguished below as "non-net reflections."

Figs. 3 and 4 show that the pair of $h = \pm 1$ reflections at $k = 7$ and 8 are, as with corresponding pairs in paramyosin, not at identical transverse locations, but are shifted slightly relative to each other in a lateral direction. (Contrary to the situation with paramyosin, however, with actin the reflection closer to the meridian is the one with smaller rather than larger k.) If
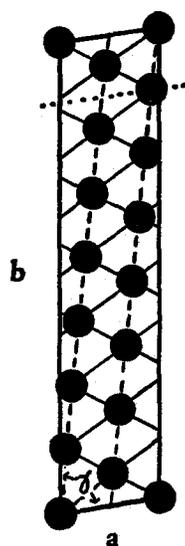

Fig. 5. The actin-net cell. Cell elements a, b, and γ are 82 A, 406 A, and 82°, respectively, for dry samples. The dotted line shows how the cell is shortened ($b' = 351$ A) if k' rather than k indices are adopted. Solid circles denote nodes, whose primitive connection is shown by light continuous lines; the outline of the non-primitive cell is heavy. Dashed lines show the nodal connection preferred for the actin "rods" in the Discussion section.

this phenomenon is taken to mean, as with paramyosin, the existence of crossed row lines, non-orthogonal to the equator, one arrives at a possible reason (along with others discussed above) as to why the reflections at higher layer lines become quite spread out transversely on the diagram.

As with paramyosin, the actin diagram can be interpreted most simply as that of a two dimensional net, in this case of the type shown in Fig. 5. The indicated cell dimensions for the net are $a \doteq 82$ A, $b = 406$ A (axial translation), $\gamma \doteq 82°$. The angle γ between a and b axes is now less than 90° because of the switch-over of relative transverse locations of the pair of reflections at $k = 7$ and 8. (The left-right orientation of both paramyosin and actin nets has been chosen so that $h = +1$ for the reflection of smaller k in each pair of

adjacent reflections on the $h = \pm 1$ row lines.) The elements a and γ were estimated from the ξ coordinates of the reflections at $k = 7$ and 8.

Note that the actin net cell is highly non-primitive, containing a large number (15) of equivalent locations (nodes). A simple primitive cell, containing nodes only at its four corners (one per cell) represents the smallest part of the structure which would be required to describe the net. Use of the non-primitive net cell has, however, the advantage of placing one cell edge along the important filament axis and of providing a more useful framework for subsequent discussion relating to macromolecular structure.

In the k indexing of Table I it has been assumed that both the net reflections and the central non-net ones belong to the same system, or at least to structures with the same axial periodicity of around 400 A. Since this need not be the case, the alternative k' indexing is possible. In this second choice a new selection rule becomes applicable:

$$h = \frac{k' - 13m}{6}.$$

No alteration of the h indices is required.

This arbitrariness of net description is not, however, as serious as might be supposed, because of a property of nets illustrated in Fig. 5. The dotted line indicates how one would cut off the larger cell with $b = 406$ A to go to the somewhat shorter one having $b' = 351$ A. Very little nodal shifting is required. Thus, it is clear that little importance attaches to the question of which net structure is adopted until one wishes to consider whether the non-net reflections have anything to do with actin-net structure, or until for other reasons the value of the b cell edge needs to be more definitely specified. The central non-net reflections unmistakably belong to a structure with b around 400 A, and the longer net cell would best fit such a period.

One can determine the width of planar net that would yield the transverse lengths of the reflections at $k = 7$ or 8, which are the only ones isolated distinctly enough on the diagrams to permit application of line-shape considerations to this problem. On the assumption that a filament width of limited size in the plane of the net is largely responsible for these diffraction line lengths, equations for a very long, thin, two dimensional diffractor were applied to densitometer traces of these reflections. It was found that a filament width comprising approximately *one* net cell was indicated. Since the non-primitive cell contains 15 nodes across its width, a filament of this type can show reflection row lines. Details of this part of the investigation are given by Cannan (1950). Estimates of the filament dimension normal to the net plane could not be made satisfactorily from lengths of meridional ($h = 0$) reflections, as was possible with paramyosin.

DISCUSSION

The Net-Helix Ambiguity

As a matter of convenience thus far the moderate-angle reflections of actin have been analyzed in terms of a planar net cell. As was shown in the similar paramyosin case, this description implies that the structure may also be interpreted as a helical arrangement of macromolecular particles.

In the helical interpretation of the net b remains the same, but the transverse cell dimension becomes πD, in which $D = 0.586\ a$, as was demonstrated in

connection with the paramyosin analysis. Here D is found to be close to 50 A, and is the diameter of the cylindrical shell through nodal centers when the cell is rolled to make vertical edges coincide in forming the helix. The *genetic* helix (describing the simplest, single coil, nodal connection) then contains 15 nodes on 7 turns along the axial period of 406 A. In forming the helix from the net, the γ angle is deformed to 90°, and the "row-line crossover" phenomenon is ascribed to the particular kind of radial distribution of matter existing about nodes.

Note that D as estimated above would *not* be the outside diameter of the helix, which may be expected to be somewhat larger than the value measured for nodal centers. There is a formal possibility that the helices might be formed from nodal patterns two or more cells in width (*cf.* the "net ambiguities" discussed by Bear, 1955), but in such cases even larger diameters would result, which seems excluded by the electron-optical information, cited subsequently, indicating the smallness of actin filament diameters.

Decision between the planar net and helix interpretations for actin is not easily made for the following reasons: (*a*) direct electron-optical resolution of nodes and their distribution in actin filaments has not been achieved because of the rather small internodal distances; and (*b*) in this diffraction study details of the "row-line crossover" phenomenon could not be examined satisfactorily very far along the $h = \pm 1$ row lines. In either case one has been unable to observe very clearly the difference which might distinguish true nets from helices, as was possible with paramyosin. While paramyosin fibrils were concluded to contain true nets, in some respects the helical interpretation is more attractive for actin.

At this stage of knowledge regarding actin, however, decision between the two interpretations is not crucial. In either case the filament is one net cell wide and has thickness of 50 to 100 A. Consequently, discussion can be based on the planar net cell, which is readily rolled into the helical model whenever required.

Macromolecular Structure

One rather expects that the fundamental macromolecular units of the actin net will be highly asymmetric, like the rods postulated for paramyosin. Inspection of the actin net (Fig. 5) shows a pronounced pair of nodal rows, each extending over two net cells along the filament axis and running at a small angle (12°) to the latter. If the actin rods follow these paths, the structure resembles the one developed for paramyosin. Close packing of two such rods across the filament allows each to be of diameter 25 to 50 A. The extent to which diffracted intensity extends from the meridian along layer lines is that expected of rods of this order of size, running nearly parallel to the filament axis (*cf.* a similar argument for paramyosin).

Existing physicochemical and electron-optical evidence is in fair agreement with this model, as far as details can be correlated at the moment. The electron micrographs of Rosza, Szent-Györgyi, and Wyckoff (1949) show that reconstituted fibrous actin forms filaments about 100 A in diameter, and when several of these are side-by-side, an oblique striation, possibly related to the intra-filament rod orientation, becomes quite noticeable. The distance from striation to striation, measured along the filament axis, is 300 A when determined electron-optically, in contrast to the 406 A suggested by the x-ray net cell. (The latter figure might be reduced to 351 A if the alternative k' indexing of the net reflections is adopted, as considered above.)

Similar electron-optical evidence of axial periodicity in actin filaments within *intact muscles* indicates figures closer to the 400 A value (Draper and Hodge, 1949; Hodge, Huxley, and Spiro, 1954). According to Hanson and Huxley (1953) the actin filaments should be seen most purely at I bands of striated muscle fibrils. Investigators have failed to note any marked change in the 400 A periodicity between A and I bands, although Draper and Hodge state that possibly it is greater at I bands than at A bands. It seems possible, therefore, that the appreciably smaller periodicity observed in reconstituted actin filaments is a result of shrinkage.

The angle which the oblique striations make with the filament axis is given by Rosza *et al.* (1949) as 20° or greater; *i.e.*, somewhat larger than the 12° suggested by the x-ray analysis. Again this discrepancy is scarcely decisive in view of distortion and resolution difficulties facing the electron-optical observations.

According to Huxley (1953 *b*) actin filaments in muscle, as observed electron-optically, have diameters of 40 A, which is below the range suggested here. Huxley's actin filaments are thin relative to the myosin ones of 110 to 140 A diameters, indeed so thin that the reliability of estimates based on photographs of stained preparations is doubtful. Huxley's identification of actin filaments in whole muscle fibrils has been questioned by Hodge (1955), but the latter's myofilaments are said to have actin cores which are also very thin.

The monomeric unit of actin is said to be approximated in shape by a prolate ellipsoid of length 290 A, width 24 A (*cf.* the summary of the situation by Tsao, 1953). A single mono-mer of this type could extend over as many as 5 nodes along each rod. On the other hand, relatively globular monomers, of linear dimensions approximating a single internodal length (55 A), would be of about the same volume and allow formation of a simpler picture of rods as linear strings of globular units.

The rod diameter is once to twice the monomer width, so that from one to four parallel actin molecules make up the rod cross-section. The x-ray data require a further property of the rod: that along a two cell length there be 15 locations of similar (identical or nearly identical) structure, corresponding to the 15 nodes along the appropriate double cell diagonal. This repetition, or near repetition, of rod structure along its axis could result from inner structure of the actin molecules, as well as from a manner of relative displacement of these along the rod. The latter type of rod structure is most simply conceived in helical models, where a rod need not be terminated in each pair of cells.

Since the nature of the wide-angle diffraction by actin is not known, one cannot offer at present any conception of the polypeptide chain configurations involved within rods. The possible large-scale helical structure mentioned above is one involving twisting of rods about each other, and not residue-scale coiling of polypeptide chains within rods or monomeric components.

Relation of Actin to Other Muscle Components

The non-net reflections make their appearance along with the ones derived clearly from the actin net in passing from the white to the tinted portions of *Venus* muscles. It does not follow, however, that both sets of reflections have common origin. The following possible relationships of these two diffraction systems are worth pointing out at this stage:

1. The origin of the non-net reflections may be a component which is chemically a part of the actin itself but superimposes a different type of large-scale structure upon the basic net-like organization. One puzzling feature of the net diffraction is the fact that the (hk) reflection $(\bar{2}1)$, corresponding to the orientation of rod axes as suggested above, has not been observed. Possibly the superimposed non-net structure is such as to favor at small angles reflection from the single cell diagnonal $(\bar{1}1)$ instead of from the expected double cell diagonal $(\bar{2}1)$. Internally twisted rods might accomplish this, and indeed the non-net reflections seem to have the characteristics of the transform of a coil of about 400 A pitch and 50 A diameter.

2. Possibly the non-net reflections result from incorporation of smaller molecular species into the actin filaments, in such a way as to accomplish the superimposition on the net of an electron density variation like that described in the last paragraph.

Perry and Reed (1947) at one time suggested that the use of alkali and acetone as "loosening" agents in the Straub method for isolating actin might indicate that in intact muscle actin may be associated with substances of a lipid nature. Tsao and Bailey (1953) favor application of butanol to extract phospholipids prior to isolation of actin, during which acetone is used to suppress liberation of tropomyosin. The present demonstration of the sensitivity of the actin net to destruction by ethanol, while possibly not requiring incorporation of lipid, rather draws one's thoughts in this direction.

The elusive "Lotmar-Picken substance," found in all types of muscle, is also of interest in this connection. Evidence of its presence is encountered accidentally on wide-angle diffraction diagrams, presumably because of liberation from normal incorporation into some part of the muscle. When free in the muscle it can be dispersed or extracted by water or benzene (Bear and Cannan, 1951; Huxley and Kendrew, 1952).

Draper and Hodge (1949) found that striated muscle fibrils, incinerated in an electron beam, retained mineral salts (presumed to be Mg and Ca salts) deposited in a 400 A periodicity along myofilament axes. Divalent metals, nucleotides (ATP, ADP), and inorganic (pyro- and poly-) phosphates are influential in determining the degree of polymerization of actin and its interaction with myosin (*cf.* Weber and Portzehl, 1952; Tsao, 1953).

3. The non-net reflections may be derived from another macromolecular component of the muscle.

According to Huxley (1953 *b*) and to Hanson and Huxley (1953) the myosin and actin filaments are distinguishable in striated muscles. According to these authors the 400 A period observed electron-optically at isotropic levels of the sarcomere would arise from actin alone. At anisotropic regions, however, myosin could also be involved. Philpott and Szent-Györgyi (1954) have shown that light meromyosin, which is in their view the contractile part of myosin, can be reconstituted into a cross-striated fibrous form exhibiting electron-optically an axial periodicity of 420 ± 25 A. Although no large-scale structure has been directly observed with heavy meromyosin, which is capable of combination with actin, molecules of this myosin fraction are described from physicochemical evidence as having a length of 435 A (Szent-Györgyi, 1953).

The electron micrographs of light meromyosin showed an outstandingly prominent and sharp band in each identity period. A sharp band of this type could produce the meridional series of consecutive diffraction orders of a 420 A periodicity observed by Huxley (1953 *a*) in living frog sartorius muscle at very small angles. Huxley's patterns were obtained with slit

collimation, so that description of transverse distribution of intensity along layer lines is not available for comparison with our results on molluscan muscle. Also, while vertebrate striated muscles definitely contain myosin, the presence of a homologous component in molluscan muscles is uncertain, though Hall, Jakus, and Schmitt (1946), in early electron-optical studies were able to extract and reconstitute, from clam muscle, filaments of dimension and appearance similar to myosin ones derived from rabbit and frog muscles. It remains possible that our non-net reflections, as well as those of Huxley's axial series which are inappropriate for the actin net, may result from components identical or homologous to myosin, or to fractions thereof.

Hodge (1955) favors, for models of striated muscle fibrils, use of a single type of filament which has a core of actin with diameter *ca.* 40 A. Between filaments or plastered thereon are particles of myosin and another as yet uncharacterized protein (that of Szent-Györgyi, Mazia and Szent-Györgyi, 1955). Of most significance in the present discussion is his postulation of interfilament bridges of tropomyosin repeated along filament axes at separations of several hundred Angstrom units.

Relationships to Muscle Physiology

The easily discernible correspondence between the present muscle diagrams and the results obtained by Astbury and Spark (1949) from films of isolated actin (see Table I) strongly indicates that the "actin-net reflections" do indeed originate from actin-containing filaments. Improved fibrous actin preparations and patterns secured recently by Cohen and Hanson (1955) provide further confirmation. The participation of other macromolecular components in the determination of major features of the net system can, therefore, be ruled out, but it remains possible that smaller molecular species may be involved in the actin-net structure.

Examination of Table I, particularly relative to reflection intensities, reveals some significant differences between whole muscle and isolated actin diagrams, and others are likely to appear after further study. Nevertheless, in our experience the actin-net diffraction obtained from a wide variety of muscles, as well as from molluscan muscles dried at various lengths (rest length ±30 per cent), remains remarkably characteristic. One gains the impression that the diffraction arises from relatively stable filaments rich in polymerized F-actin.

On the other hand, actin does not remain aloof from the physiological activity of muscle. Air-dried muscles, such as were studied in this investigation, may be presumed to be in a state of rigor; *i.e.*, to be ATP-poor. Under these conditions actin and myosin are often considered to be associated. Why then does the actin structure remain apparent in the diffraction pattern?

The results of Huxley (1953 *a*) provide a partial answer to this question for striated muscle. Huxley found that x-ray evidence of actomyosin formation is most readily apparent at the equator of the diffraction field but it is more elusive near the pattern meridian. One expects axially projected structure of a filamentous component or combination to be represented near the meridian

and the details of transverse organization to appear near the equator. Huxley's observations suggest that the interaction between actin and myosin filaments or particles may be largely at surfaces between them, drawing them into union transversely in the muscle fibril but not excessively disturbing their separate internal structures and orientations.

While the actin component of a muscle is fairly readily recognizable by means of diffraction, this cannot be said as yet for other macromolecular constituents (except paramyosin, when present). Further study of the non-net reflections at very small diffraction angles will be important for recognition of the state of these other substances. Myosin (or its homologues or fractions) is most likely to be involved here. Diffraction examination of the isolated components will be useful in providing criteria for the interpretation of the observations on whole muscle.

It is probably of considerable significance that most of the macromolecular constituents of muscles (actin and both meromyosins, as well as Hodge's tropomyosin bridges) may possess or contribute to spacings or dimensions near 400 A. Certainly one can no longer be satisfied with a single source for periodicities of this size. Although diffraction evidence of physiological state and function in muscles has long been difficult to obtain, it is now becoming apparent why this has been so and what kinds of information are to be sought.

SUMMARY

From analysis of moderate- to small-angle x-ray diffraction patterns, in the light of similar experience with paramyosin, has been derived the following description for the structure of actin-rich filaments in "tinted" portions of the adductor muscle of the clam, *Venus mercenaria:*

1. Some 11 diffraction maxima, widely streaked along layer lines and occurring at moderate diffraction angles (spacings 7 to 60 A) appear to be accounted for as (hk) reflections of a net whose cell elements are, for dry material: $a \doteq 82$ A, $b = 406$ A (filament axis identity period), and $\gamma \doteq 82°$ (angle between a and b axes). These reflections follow a selection rule which indicates that the net cell is non-primitive and contains 15 equivalent locations (nodes) arranged as shown in Fig. 5. An alternative net has $b' = 351$ A and 13 nodes per cell.

2. Another interpretation rolls the net into a large-scale helix and places the 15 (or 13) nodes along 7 (or 6) turns of a helical locus projecting 406 (or 351) A along the filament axis. Whether considered to be built of planar-net or helix-net cells, the individual filament contains a single cell width transverse to its axis. Transverse filament dimensions are, therefore, in either case similar (50 to 100 A).

3. Consideration of existing electron-optical, physicochemical, and x-ray diffraction data regarding isolated actin suggests that the net cell is built of

rods, each containing in cross-section from one to four actin molecules which run parallel to or twisted about rod axes that extend at 12° to the filament axis along the ($\overline{2}1$) diagonals of the cell. Depending on monomer shape, 2 to 15 monomers furnish length to reach across two cells, and the actin molecules are built into each rod in such a way as to repeat (or nearly repeat) structure 15 (or 13) times along the double cell length. Further details of intra-rod structure cannot be suggested because of lack of wide-angle diffraction information.

4. The actin system is sensitive to treatment of the muscle with ethanol. Concentrations of 5 per cent or greater abolish the net reflections. Other solvents—water, benzene, ether, pyridine, acetone—do not alter the pattern materially.

5. Two other reflections, occurring at the first and second layer lines of an axial periodicity of about 400 A, do not clearly belong to the actin-net system. They represent either a superstructure built upon the filaments by parts of the actin molecules themselves or by incorporated other molecular species, or they arise from an additional macromolecular component (possibly myosin, or its homologues or fractions) of similar axial periodicity.

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