REVIEWS OF MODERN PHYSICS VOLUME 31, NUMBER 2 APRIL, 1959

45
Fibrous Proteins of Muscle*

ALAN J. HODGE

Department of Biology, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

A FULLY coherent and integrated picture of the structure of muscle in terms of its various components and the changing relationships accompanying or responsible for contraction is still a long way off, as becomes evident later. An adequate description of muscle contraction requires knowledge sufficient to answer questions such as the following: First, which are the components of the myofibril actually participating in the contraction? Second, how does the structure of the individual components and their interrelationships one with another change during shortening? Third, how is the mechanical work produced at the expense of free energy derived from the hydrolysis of adenosine triphosphate (ATP)? The first of these questions is, at present, the only one which can be answered with any certainty. Although many models have been proposed for the contraction process, the physical changes accompanying contraction remain a subject for speculation, largely because of the difficulty in applying appropriate physical methods, such as x-ray diffraction and optical rotation, to the problem. The electron microscope has been employed with striking success in elucidating the details of muscle fine structure but, because of certain inherent limitations, it has been much less useful in following the structural changes accompanying contraction. A final solution, it would seem, can be accomplished only by a careful and rigorous synthesis of data obtained from light and electron microscopy, x-ray diffraction, optical rotation, and other physical and chemical techniques.

Before passing on to a consideration of the individual muscle proteins, it seems appropriate to review briefly some aspects of the progress which has been made since Engelhardt and Ljubimova[1] demonstrated the ATPase activity of "myosin." Later, Szent-Györgyi and his collaborators showed that myosin is a complex of two proteins, actin and L-myosin, and that the association of these components is influenced by the presence of ATP.[2,3] This discovery led to an extended period in which the mechanical and other properties of actomyosin threads were intensively studied. The discovery and development of the glycerine-extracted model system provided further stimulation for the study of artificial model systems in general. Further progress in narrowing the gap between intact muscle and the various extracted actomyosin systems was made by the discovery of substances able to influence contraction in one way or another. Of these, perhaps the best-known is the "relaxing factor" of Marsh.[4] The more-refined physicochemical methods developed in recent years have been applied to advantage in characterizing the various muscle proteins, but they have been relatively unsuccessful in elucidating the molecular changes accompanying contraction phenomena. Furthermore, it is difficult to extrapolate from the behavior of macromolecules in solution to their behavior in a complex paracrystalline multicomponent system like the myofibril. There exists an urgent need for the development of techniques which will allow the determination of configurational changes both at the molecular and macromolecular levels during contraction in intact muscle. X-ray diffraction is the only method currently applicable to this problem, and its application is fraught with very great technical difficulties, stemming largely from the short duration of a twitch and from the very high source intensities required for an adequate diffraction record at low angles. The application of optical-rotation methods appears to be an attractive possibility provided that the physical difficulties arising from the small dimensions and high degree of orientation of the contractile components can be overcome.

Following the pioneering work of Astbury in classifying the fibrous proteins into the α class (kmef group, now known to possess the α-helical configuration[5–7]), and other classes which need not be considered here, Bear[8] showed that the x-ray diffraction patterns of various types of muscle fall into two distinct groups as judged by their low-angle diffraction patterns: (1) the Type I or paramyosin pattern corresponding to an axial repeat of 725 A found only in certain invertebrate muscles possessing the so-called "catch mechanism"; and (2) the Type II diffractions corresponding to axial spacing of about 400 A which are found in all muscles. As becomes evident later, this classification based on x-ray spacings is probably far more reliable as a criterion than some of the biochemical properties such as solubility and amino-acid composition, which have been employed in recent years as bases for nomenclature of the muscle proteins. At first, it was thought that this 400-A periodicity arose from a single component. However, as is seen, x-ray diffraction studies on purified components of striated muscle indicate that most, if not all of them, exhibit periodicities of about 400 A. Furthermore, the macromolecules of many of these Type II components have lengths of about this magnitude, and electron micrographs of myofibrils frequently show an axial spacing of similar dimension. It seems very likely, therefore, that, in the myofibril, the various

* This paper contains descriptions of original work by the author (on tropomyosin and paramyosin) which was aided by a research grant, E-1469 (C1), from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

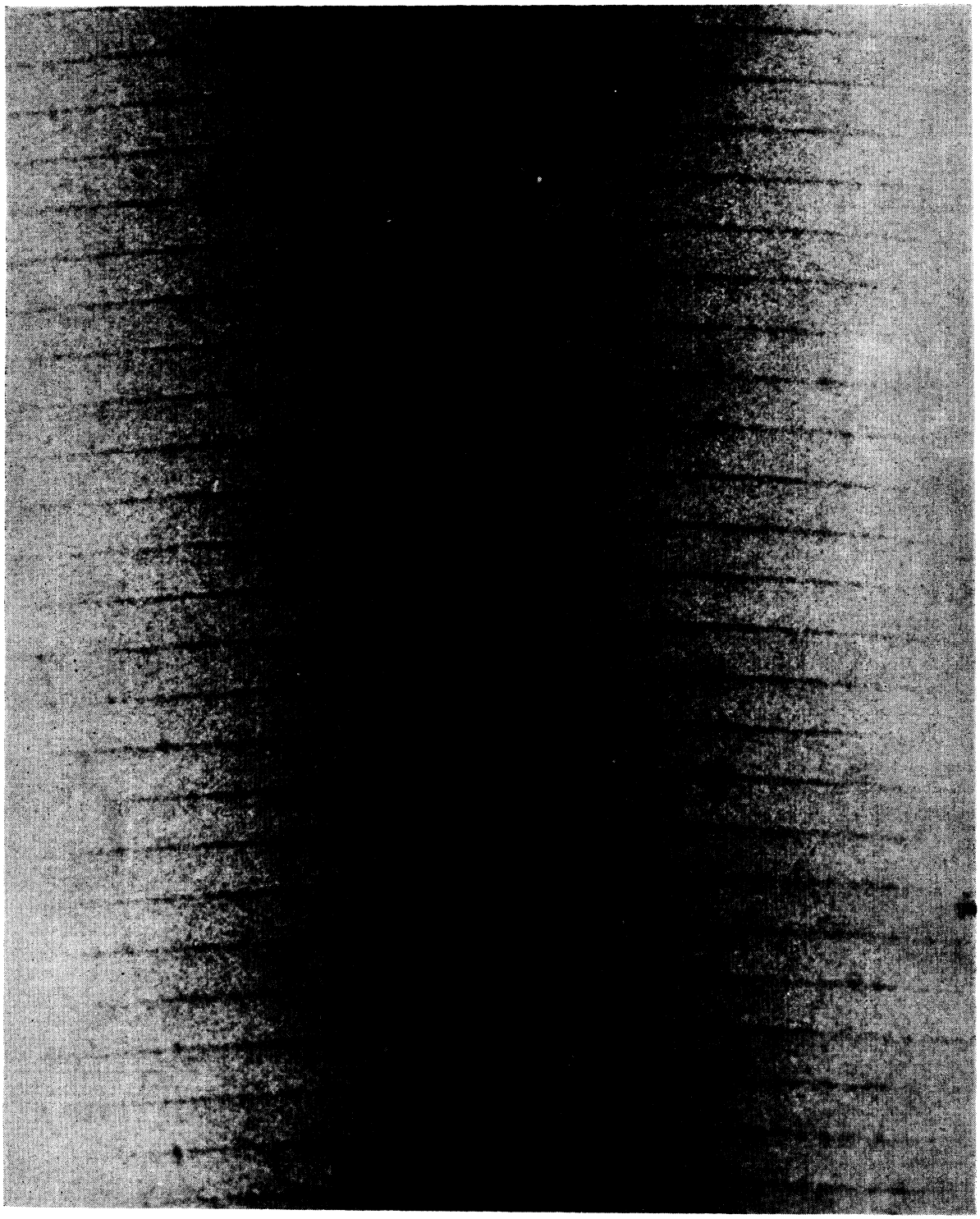

FIG. 1. Crystal of LMM showing the sharp axial banding observed in the electron microscope in the absence of an "electron stain." The very dense transverse striations spaced about 420 A apart are probably owing to binding of salts by the end regions of the LMM molecules [from D. E. Philpott and A. G. Szent-Györgyi, Biochim. et Biophys. Acta **15**, 165 (1954)]). ×200 000.

components interact with one another in orderly fashion by means of reactive sites repeating at distances of about 400 A along the fiber axis.

The results of Huxley and Hanson[9] indicate that intermolecular rearrangement is an important part of the mechanism of contraction in striated muscle. How-

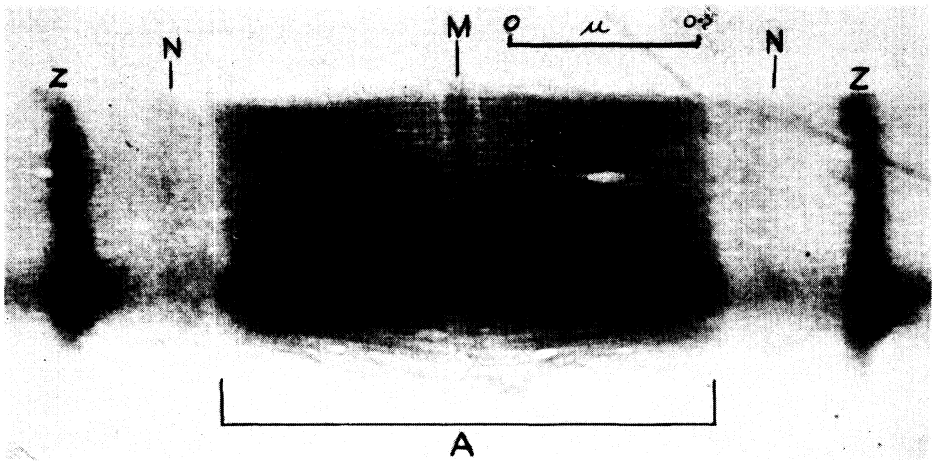

FIG. 2. Electron micrograph of a myofibril isolated from formalin-fixed toad muscle and stained with phosphomolybdic acid, showing the regular 400-A cross-striation in the form of fine, transversely oriented, dense lines [from M. H. Draper and A. J. Hodge, Australian J. Exptl. Biol. Med. Soc. **27**, 465 (1949)]. ×64 000.

ever, the results obtained so far do not rule out the possibility that configurational changes are involved as well—the results of detailed correlative x-ray diffraction and other measurements must be awaited before any definite conclusions can be drawn, and in any case, the results obtained with striated muscle are not necessarily applicable to all types of contractile systems.

ACTIN

This component of the actomyosin complex was isolated by Straub.[10] It appears to be a necessary component of the contractile system in muscle and comprises 15 to 20% of the structural proteins in rabbit striated muscle. An outstanding characteristic of actin is its ability to undergo polymerization (the $G-F$ transformation) to form well-defined fibrous elements, a process accompanied by the dephosphorylation of strongly bound ATP. The monomeric form of actin is one of the most difficult muscle proteins to characterize from a physicochemical point of view since the presence of salt favors the formation of the fibrous form. However, it seems likely that the monomeric unit has a molecular weight of about 60 000 (Table I, from work by Cohen and A. G. Szent-Györgyi[11]) with physical dimensions rather more uncertainly defined. The work of Astbury[22,23] and of Selby and Bear[24] indicates that actin exhibits an axial-repeat period of about 400 A in conformity with the other proteins of vertebrate striated muscle and of smooth muscle, both vertebrate and invertebrate. Actin has a pronounced tendency for complex formation with myosin, but is devoid of enzymatic properties.

TABLE I. Dimensions of fibrous-muscle proteins [from C. Cohen and A. G. Szent-Györgyi, paper read at International Biochemistry Congress, Vienna (1958)].

	M.W.	Est. length (A)	Est. axial ratio
Actin	57 000[12]–74 000[13]	290[13] ?	12[13] ?
Myosin	450 000[14–17]	1600	50
HMM	230 000[18]–330 000[19]	400[18]	15–20[18]
LMM	96 000[18]–140 000[19]	550[18]	30–40[18]
LMM fr. 1	110 000 –120 000		30–40
Tropomyosin	53 000[20]	385[20]	25[20,16]
Paramyosin	134 000[21]	1400[21]	80[20]

MYOSIN

Myosin is the major constituent of most types of muscle, e.g., it accounts for 55 to 60% of the structural proteins of rabbit skeletal muscle. It has ATPase activity which is calcium activated and magnesium inhibited. It is a globulin, precipitating at low ionic strength, and can be extracted from muscle at neutral *p*H with solutions of ionic strength higher than about 0.6. Characterization of the myosin molecule has been a subject of controversy for many years, but there seems now to be general agreement that the molecule has a length of about 1600 A, an axial ratio of about 50, and a molecular weight of about 420 000 (Table I).

Brief exposure of myosin solutions to proteolytic enzymes such as trypsin,[25–27] chymotrypsin,[28] and subtilizin[29] degrades the myosin molecule into two fairly well-defined components, heavy meromyosin (HMM) and light meromyosin (LMM). The well-known properties of the myosin molecule are divided between these fragments. LMM behaves physically very much as does myosin. It precipitates at low ionic strength and has a molecular weight of about 100 000. However, it has no ATPase activity and does not combine with actin. On the other hand, HMM has all of the ATPase activity of the myosin from which it was derived, combines with actin in the same proportions as does myosin, but differs from myosin in being soluble in solutions of low ionic strength. Reported molecular weights range from 230 000 to 320 000 (Table I). HMM, like myosin, does not form ordered precipitates. LMM, on the other hand, under appropriate conditions, forms highly ordered needle-shaped crystals exhibiting a very striking axial period of about 420 A in the electron microscope, even in the unstained condition, as shown in Fig. 1 from work by Philpott and Szent-Györgyi.[30] It seems very likely that

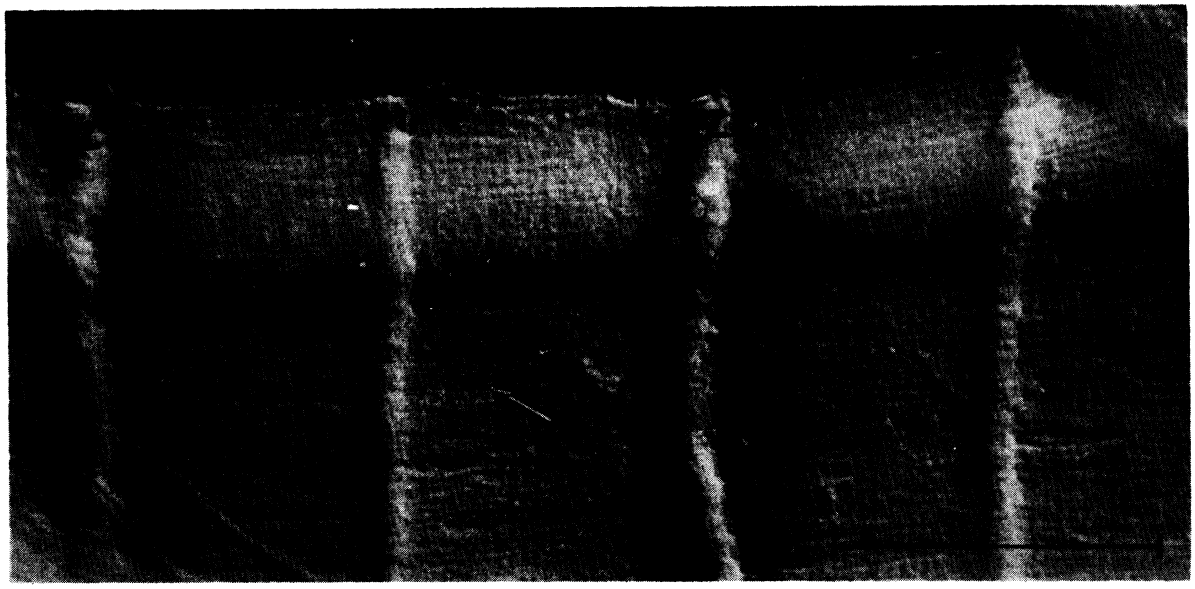

FIG. 3. Semicontracted myofibril of toad muscle, shadowed with platinum, showing the regular axial period apparently associated with the presence of transverse bridges (arrow) [from M. H. Draper and A. J. Hodge, Australian J. Exptl. Biol. Med. Soc. **27**, 465 (1949)]. ×45 000.

the fine cross-striations evident in these crystals arise from a selective binding of inorganic ions at specific sites located about 400 A apart (possibly at the ends of the LMM "molecules"). This result is in good agreement with the fact that isolated myofibrils, when shadow-cast or stained with phosphotungstic acid (PTA) or phosphomolybdic acid, exhibit a sharp and regular cross-striation[31,32] (Figs. 2 and 3) with an axial period of about 400 A in the relaxed condition, and it agrees with the evidence obtained by Draper and Hodge[33,34] concerning the distribution of bound mineral in myofibrils after electron-induced microincineration in the electron microscope.† These authors found that the mineral residue from well-washed myofibrils obtained from formalin-fixed muscles is present as fine cross-striations with an axial spacing of about 400 A (Fig. 4), a result suggesting that the various inorganic and organic components of muscle interact one with another by means of reactive sites or groups located about 400 A apart. Further evidence[36] for this view is provided by the regular cross striations observed in thin sections of muscle (Fig. 5). There is in addition, some electron-microscopic evidence to suggest that this fundamental period may shorten during contraction,[37] a result which, if borne out by further investigation, strongly suggests that contraction must involve a configurational change in at least some of the macromolecular components of the myofibril. On the other hand, the sliding filament model, in which contraction is achieved by progressive interdigitation of actin and myosin filaments, does not require such configurational changes, and it should be noted that no changes have so far been observed in the wide-angle x-ray pattern of muscle during contraction. However, such configurational changes

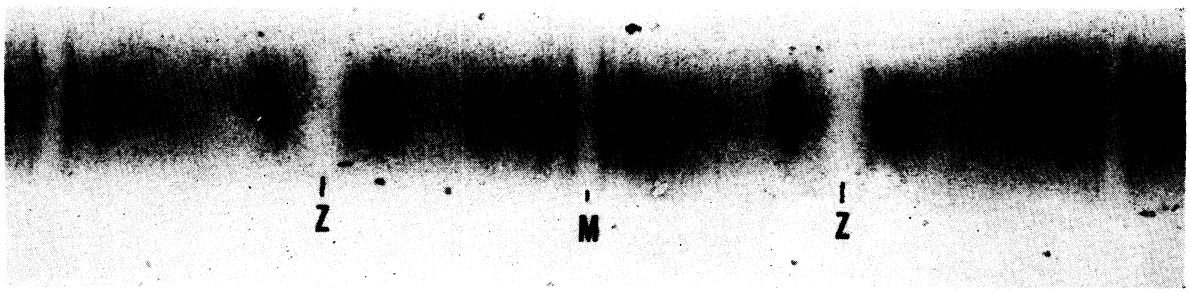

FIG. 4. Formalin-fixed rabbit myofibril after electron-induced microincineration in the electron microscope to illustrate the distribution of the bound mineral residue within the sarcomere in the form of regularly spaced, fine transverse striations spaced about 400 A apart [from M. H. Draper and A. J. Hodge, Nature **163**, 576 (1949)]. ×30 000.

† The work of Leisegang[35] provides a rationale for the microincineration of organic material induced by high beam intensities in the electron microscope.

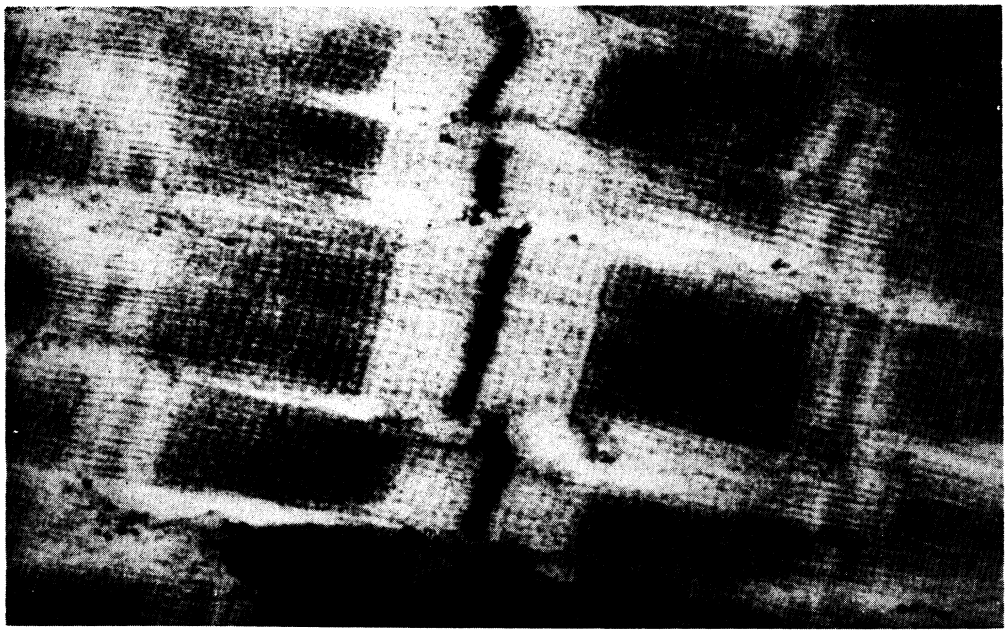

FIG. 5. Thin longitudinal section of rabbit muscle fixed in buffered OsO_4 and stained with PTA, showing the regular cross-striation present in all bands of the sarcomere [from A. J. Hodge, H. E. Huxley, and D. Spiro, J. Exptl. Med. 99, 201 (1954)]. ×40 000.

would be difficult to detect if, as seems possible, a sequential shortening of small contractile units (perhaps 400 A long) is involved in the mechanism of contraction.

A number of recent experimental investigations point toward the rather disquieting possibility that the myosin "molecule" may be an artifact of the extraction methods used to isolate this protein. Thus, although it has so far proved necessary to employ proteolytic enzymes such as trypsin, chymotrypsin, and subtilizin in order to obtain the meromyosins from preparations of myosin, there is as yet no convincing evidence to indicate that the splitting of peptide bonds is necessary for the liberation of the meromyosins from the parent macromolecule. Middlebrook[29] was unable to demonstrate the presence of C-terminal groups in the ratios expected from the known specificities of trypsin and chymotrypsin. Furthermore, the degradation of the meromyosins by these enzymes is a much slower process than their production from the parent macromolecule, a result indicating the presence of a highly sensitive region within the myosin molecule, and the results of turnover-rate studies indicate a metabolic independence of the two meromyosins. Thus, Velick[38] has shown that the turnover rate of phenylalanine is about

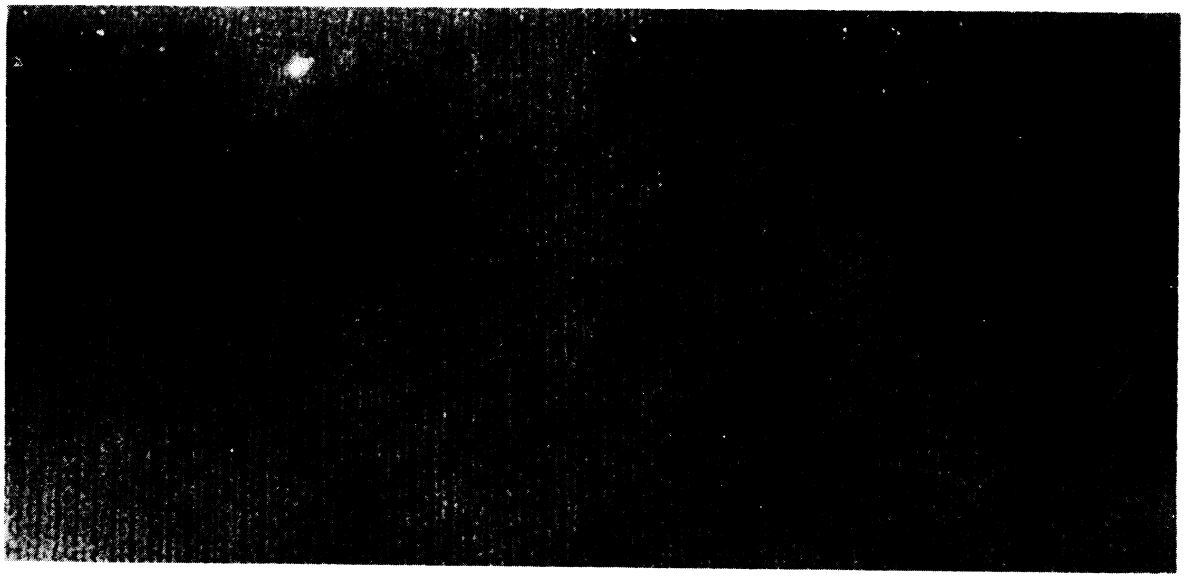

FIG. 6. Electron micrograph of a crystal of rabbit tropomyosin deposited on a supporting film and stained with PTA in *p*H 4.2 phosphate buffer. The main spacing is about 200 A, and a definite intermediate line is present. ×125 000.

FIG. 7. Crystal of rabbit tropomyosin, stained with PTA, showing the crossed-grid appearance characteristic of many of these crystals in electron microscopic preparations. ×70 000.

five times more rapid in LMM than in HMM. Similarly, Schapira *et al.*[39] have observed that the incorporation of glycine is more rapid for LMM than for HMM. These results, on their face value, may be interpreted as indicating that the meromyosins represent precursors of the myosin macromolecule. However, there is also evidence derived from careful experiments with fluorescent antibodies to suggest that at least a part of the meromyosins in the myofibril are distributed independently of each other and in a highly characteristic pattern. This is discussed later. If nothing else, the sum total of the foregoing results must sow a seed of doubt concerning the almost universally accepted assumption that myosin *per se* is a well-defined macromolecular species which by some relatively simple interaction with other species such as actin is able to bring about the phenomenon of contraction.

TROPOMYOSIN

This protein component, which is universally distributed in small concentrations in a wide variety of muscles, was discovered by Bailey[40] and is remarkable in that it is the first and probably the only fibrous protein thus far obtained in a truly three-dimensional crystalline form. The crystals of tropomyosin are platelike and have an unusually high degree of hydration (80 to 90%). This latter property probably results from the fact that tropomyosin, unlike many of the fibrous proteins of muscle (which tend to form one-dimensional paracrystalline arrays), exhibits a strong tendency for the formation of three-dimensional crossed-grid net-

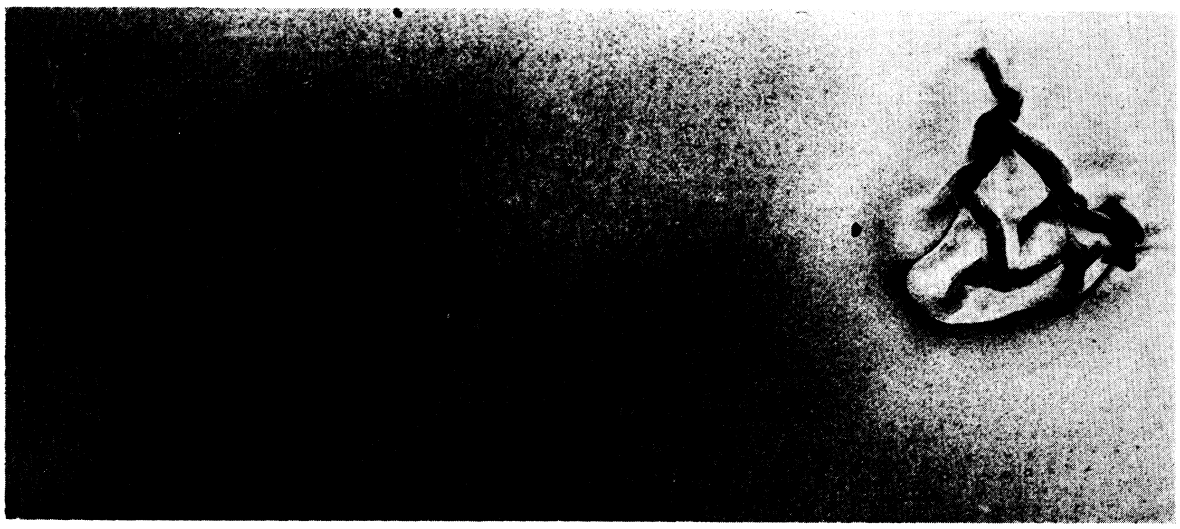

FIG. 8. Rabbit tropomyosin crystal from an ammonium-sulfate suspension mounted on the supporting film without washing or staining. The points and lines of high density probably correspond to regions where salt is accumulated. ×135 000.

works. A crystal of tropomyosin can, in fact, be regarded as a very highly ordered gel. Tropomyosin has no enzymatic activity and, as its name implies, was at one time thought to be a precursor of myosin itself. However, there is no substantial evidence for this view, and indeed, the role played by tropomyosin in muscle function remains a subject for speculation.

Tropomyosin, the water-soluble component originally defined by Bailey,[40] appears to be present in small amounts in all muscles (e.g., it accounts for about 4 to 5% of the fibrous proteins of rabbit striated muscle) and is present even in those invertebrate muscles that exhibit Type I low-angle diffraction patterns (i.e., those that have paramyosin as well as the actomyosin system). It is of interest to note that the tropomyosin molecule with a molecular weight of about 53 000 (Table I), has a length of about 400 A, as estimated from physicochemical evidence, a result in keeping with the lengths of all of the other components and subunits in the Type II system so far described.

Examination of tropomyosin crystals in the electron microscope in this laboratory has proved instructive and the spacings observed appear compatible with physicochemical estimates of the molecular length. A correlation with low-angle x-ray diffraction is currently being carried out.[41] The results obtained when a preparation of small tropomyosin crystals is placed on a grid, stained with buffered phosphotungstic acid (*p*H 4.2) or allowed to dry without staining are illustrated in Figs. 6–9. A frequently observed pattern is one comprising striations spaced about 200 A apart (often with intraperiod lines) or a crossed-grid network with periods of the same magnitude in two directions (Figs. 6 and 7). In unstained crystals (Fig. 8), the periodic structure appears rather as a pattern of dots, strongly suggesting that inorganic ions are occluded or bound at, or near, the sites of interaction of the long thread-like macromolecules arranged in an ordered gel structure. The square two-dimensional pattern with spacings of about 400 A (Fig. 9) affords yet another example of the remarkable capacity of tropomyosin to form open, ordered network structures, a property which may well have some significance in relation to the transverse bridges known to connect the longitudinal filamentous elements in striated muscle at regular intervals along the fiber axis. The regular outlines of the tropomyosin crystals become evident if they are fixed, embedded, and sectioned by conventional procedures (Figs. 10 and 11). However, dimensional analysis is rather more uncertain than in the cases already mentioned, since it is technically difficult to define the plane of the section in relation to a particular crystallographic plane. Nevertheless, the three-dimensional ordered net structure of the crystals is often clearly discernible (Fig. 12). This type of net structure is reminiscent of that observed in collagenous tissues such as Descemet's membrane[42] and in the pharyngeal structures of certain protozoa.[43] It may represent an important structural principle in ordered biological systems and may not be irrelevant to the process of contractility itself.

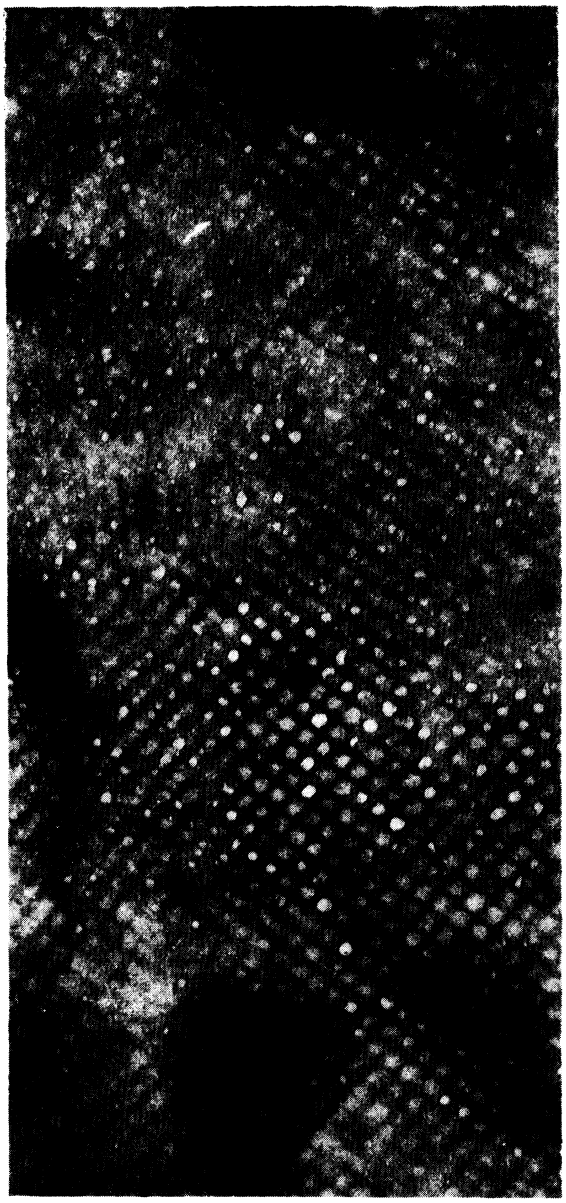

FIG. 9. Open, two-dimensional, net structure observed in a rabbit tropomyosin crystal preparation. The spacing of about 400 A corresponds to the length of the tropomyosin molecule as determined by physicochemical techniques (Table I). ×75 000.

As has been seen, there exists a rationale for an orderly interaction of the muscle components so far described in terms either of their lengths being about 400 A or of there being subunits of this length. It seems clear, therefore, that the total x-ray pattern (both meridional and equatorial) of whole muscle must contain information concerning the precise state of interaction of the macromolecular components for any given state of the contractile system, and offer the

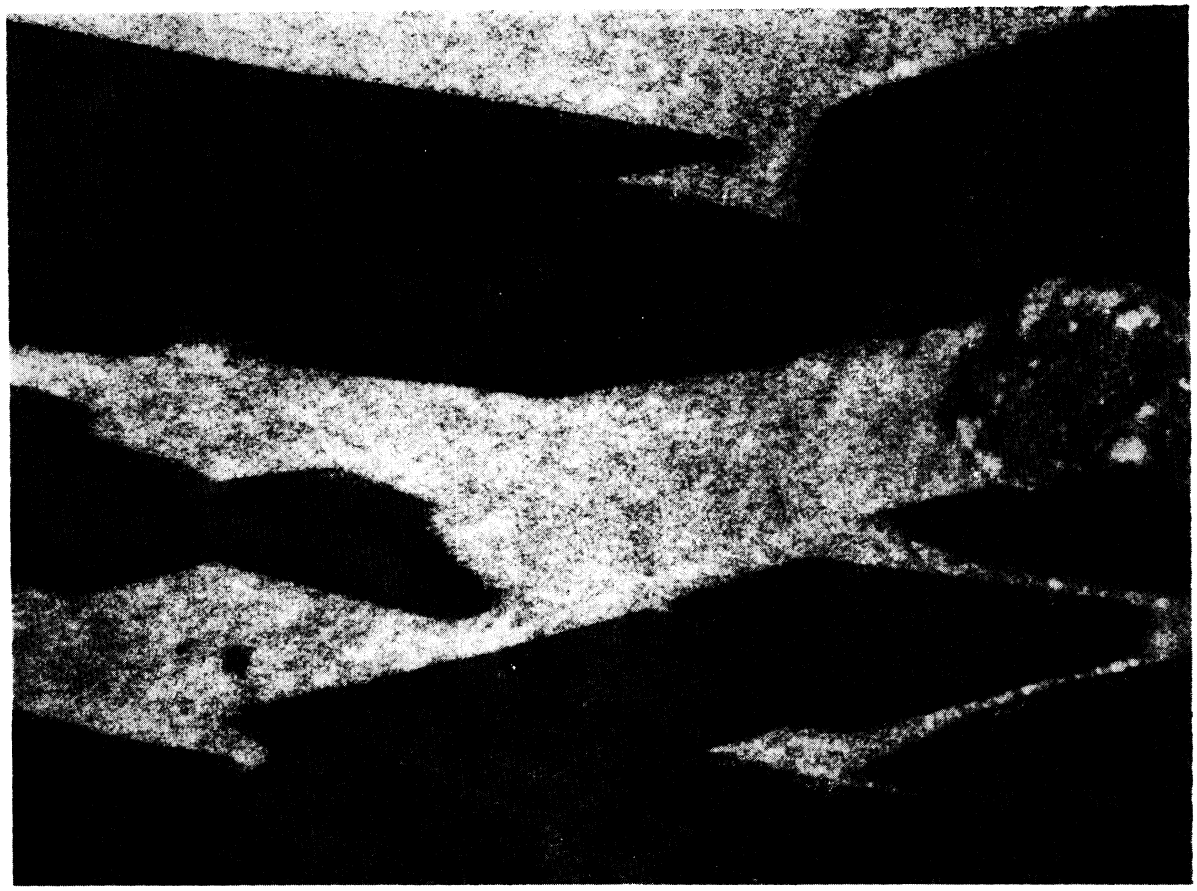

FIG. 10. Electron micrograph of a thin section of rabbit tropomyosin crystals, fixed in buffered, osmium-tetroxide solution, stained with PTA, and embedded in *n*-butyl methacrylate. Note the regular outlines of the crystals and the precise cross-striation corresponding to a spacing of about 200 A. ×20 000.

possibility of following the physical changes accompanying contraction. However, as already noted, there are severe technical problems to be overcome in any such investigation.

PARAMYOSIN

The term paramyosin was introduced by Hall *et al.*[44] to describe a major component of certain specialized molluscan and annelid muscles (Table II),[45] which possess the ability to maintain a rigor-like contracture for long periods of time and which were described in the older literature as having a "catch mechanism." It is this protein that is predominantly responsible for the Type I low-angle diffraction pattern obtained by Bear[8] from these muscles, a highly characteristic pattern comprising reflections corresponding to a fundamental repeat period of 725 A, with every fifth reflection highly accentuated. The pattern can be interpreted formally (see Bear and Selby[46]) in terms of a net structure of the type shown in Fig. 13. "Paramyosin fibrils," which are easily isolated in native form from the adductor muscles of certain marine mollusks such as the clam, *Venus mercenaria*, exhibit a characteristic band structure with a period of 145 A in the electron microscope, together with a spot pattern, the symmetry of which is such that the true repeat period of this composite pattern is 5×145 A=725 A (Fig. 13). Since this spot

TABLE II. Relative intensities of the myosin and paramyosin x-ray diffraction systems exhibited by various muscles [from F. O. Schmitt, R. S. Bear, G. E. Hall, and M. A. Jakus, Ann. New York Acad. Sci. **47**, 799 (1947)].[a]

Muscle	Myosin diffractions	Paramyosin diffractions
Mytilus adductor	+	+++++
Venus adductor, w	++	++++
Anodonta adductor, w	++	+++
Anodonta adductor, t	+++	+++
Mya adductor	+++	+++
Venus adductor, t	++++	++
Pecten adductor, w	++	++
Phascolosoma retractor	+	+
Dog retractor penis	+	...
Pecten adductor, t	+	...
Thyone retractor	+	...
Frog sartorius	+	...

[a] Rough visual estimates of the relative intensities of the myosin and paramyosin diffractions on the patterns of the various muscles are indicated by +; w and t refer, respectively, to the "white" and "tinted" components of the muscles which possess them.

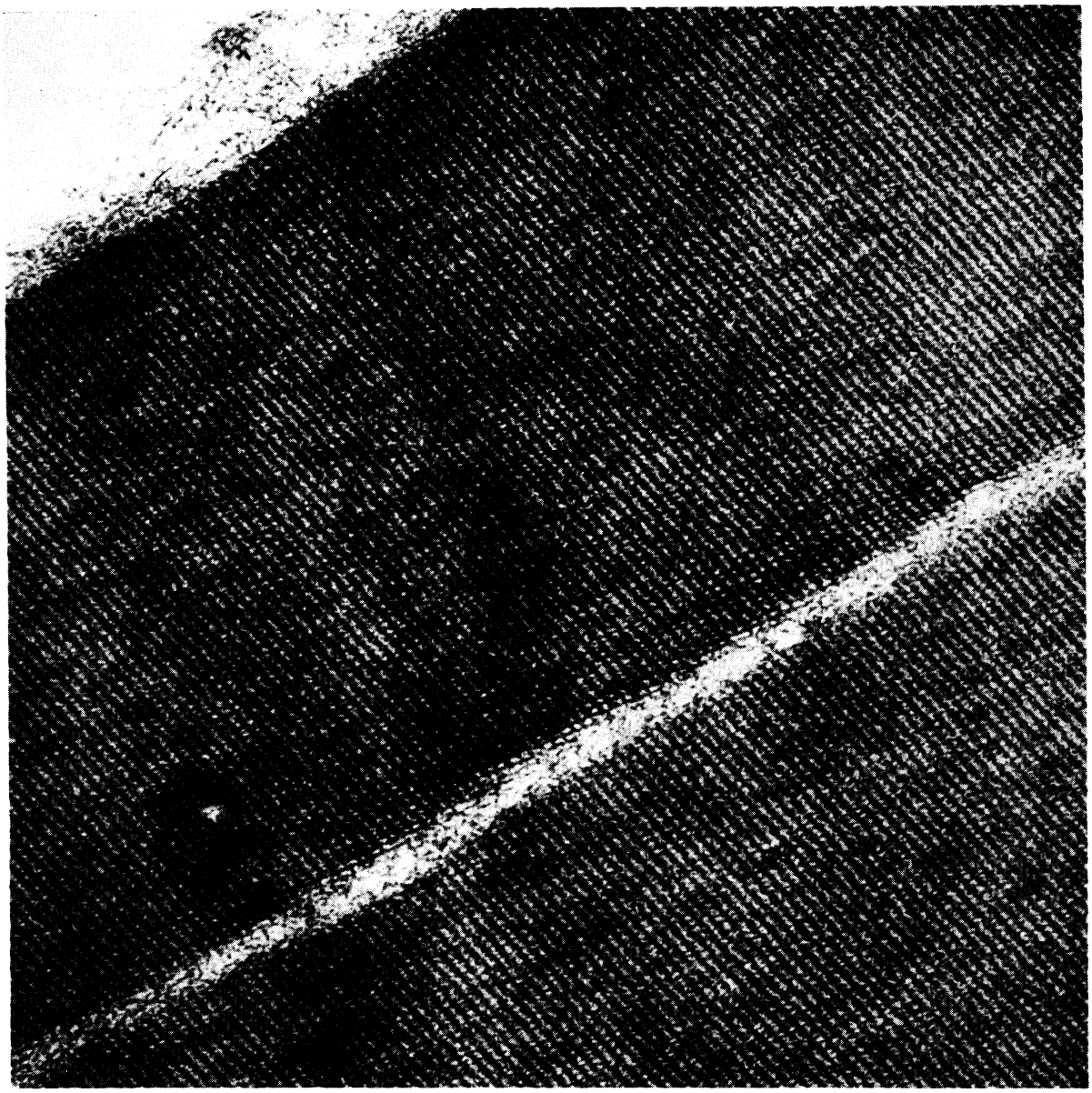

FIG. 11. Higher magnification view of two tropomyosin crystals in the same preparation as Fig. 10, showing the regular open-network lattice characteristic of these crystals. ×95 000.

pattern is frequently absent in these native isolated fibrils, and because the major component of these fibrils can be extracted and reconstituted into fibrils in which no spots are visible but which exhibit band patterns with fundamental repeat periods of 725 A (Fig. 16), it seems likely that the spots reflect the presence of one or more additional components within the fibrils. In any case, it is proposed here to restrict the term paramyosin to the major component of these native fibrils. A composite structure for the native paramyosin fibrils is further indicated by some recent work[47] in which transverse sections of adductor muscle show that the fibrils are built up of a number of thin layers.

In 1952, the author[48,49] found that paramyosin could be quantitatively precipitated from acid solutions in the form of ordered tactoidal fibrils with an axial period of about 1400 A and with symmetrical intraperiod band structure, (Fig. 14) rather than the "polarized" band structure characteristic of the native 725-A period. At that time, Schmitt *et al.*[50] were in the process of analyzing the band structures of the newly discovered segment long-spacing (SLS) and fibrous long-spacing (FLS) forms derived from soluble collagen (asymmetric and symmetric band patterns, respectively), and had deduced that the collagen macromolecule was probably four times the length of the axial period (*ca* 700 A)

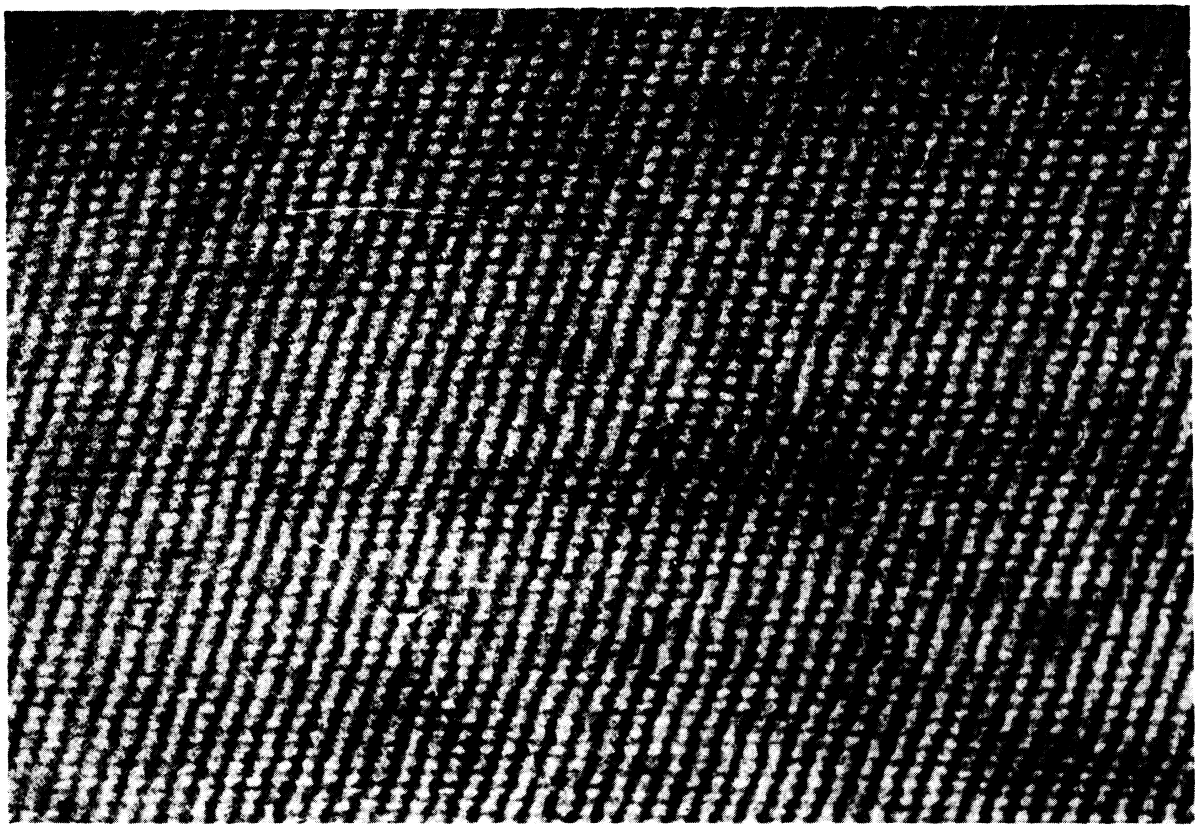

FIG. 12. Thin section of a rabbit tropomyosin crystal showing the characteristic open-network lattice. This type of structure probably accounts for the very high degree of hydration of the tropomyosin crystals. ×190 000.

displayed by native-collagen fibrils. According to their concept, the SLS forms arose by a parallel packing of the macromolecules with like ends in register, the FLS by an antiparallel packing, thus accounting for the symmetrical band structure of FLS. Consequently, by analogy with the collagen picture, it seemed likely that the macromolecules of paramyosin were about 1400 A long, and that they were packing in antiparallel array to form an FLS-type banded structure. This prediction was borne out[48,49] by the results of sedimentation, diffusion, viscosity, and light-scattering investigations, which were necessarily rather crude because of the low ionic strengths required to keep the protein in solution under these acid conditions. However, the data were sufficiently good to indicate the presence in the solutions of particles with lengths between 1200 and 1500 A and with axial ratios of about 70. Thus, the combined electron-microscopical and physicochemical evidence established the length of the paramyosin macromolecule (or at least of the kinetic unit in solution) with some certainty as being about 1400 A. The more recent and more accurate measurements of Kay,[21] carried out on paramyosin in neutral solution at high ionic strength, appear to confirm this value for the length of the paramyosin macromolecule.

Bailey[51] obtained paramyosin in purified form, and, on the basis of similarities in the amino-acid compositions of tropomyosin and paramyosin, was led to call the latter "insoluble tropomyosin."[52] Similarly, Kominz *et al.*[53] refer to paramyosin as "tropomyosin A" to distinguish it from "tropomyosin B" (i.e., the original tropomyosin of Bailey). This profusion of nomenclature seems unwarranted, and it would seem advisable at the present time to retain the term paramyosin, since there exist definite differences in solubility properties, crystalline form, and x-ray and electron-microscopic periodicities which clearly differentiate this protein from the original water-soluble tropomyosins of Bailey. Paramyosin has no enzymatic activity, according to Szent-Györgyi,[54] does not combine with actin, and there is as yet no evidence to indicate a possible function for it in relation to the specialized properties of the "catch" muscles.

A remarkable variety of fibrous structures can be formed from paramyosin solutions under various conditions of *p*H and ionic strength. Those so far obtained include fibrils with axial periods of *ca* 70, 145, 725, and 1800 A. (This last, together with one of 360 A, was first observed by Locker and Schmitt.[55]) In the case of collagen, it can be shown that the native type, 700-A

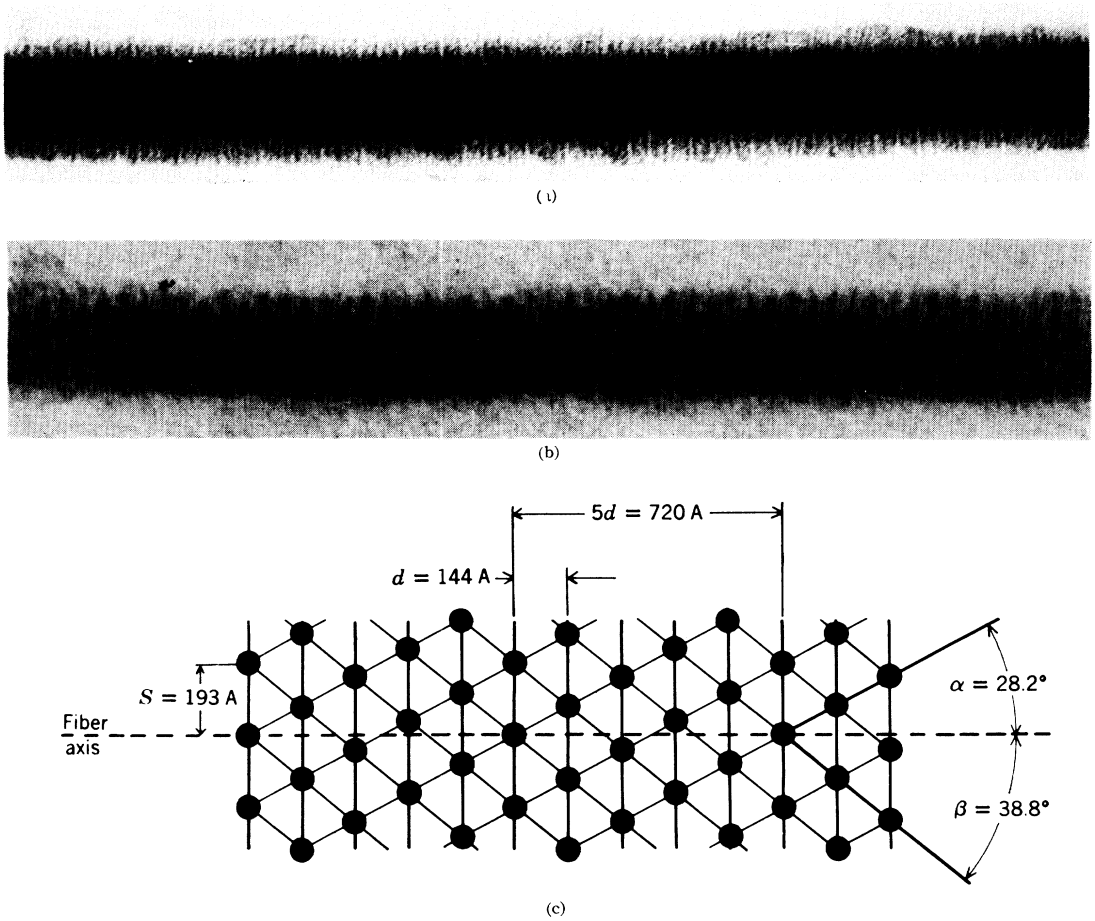

FIG. 13. Native "paramyosin fibrils" isolated from the adductor muscles of *Venus mercenaria* in dilute salt solutions. These usually show (a) a transverse cross-striation with an apparent period of 145 A,[48,49] (b) a pattern consisting of the 145-A cross-striation and a superposed dot pattern of symmetry such that the true repeat period is 5×145 A=725 A [from F. O. Schmitt, R. S. Bear, C. E. Hall, and M. A. Jakus, Ann. N. Y. Acad. Sci. **47**, 799 (1947)]. (c) shows the node pattern derived from electron micrographs. This formal net structure is in good agreement with the results of low-angle x-ray diffraction studies [from C. E. Hall, M. A. Jakus, and F. O. Schmitt, J. Appl. Phys. **16**, 459 (1945)].[8] (a) and (b) ×190 000.

periodicity can be obtained by a parallel packing in which the tropocollagen macromolecules (2800 A long) are staggered with respect to one another by one-quarter of their length. The key to such a synthesis of band structure is the availability of the SLS band structure, which is in effect a "fingerprint" of the tropocollagen macromolecule. Attempts to produce segment-type structures from paramyosin solutions have so far proved unsuccessful, but it seems likely that parallel, antiparallel, and staggered arrangements involving axial displacements of one-tenth the molecular length (i.e., 1400 A/10) or integral multiples thereof could explain the various band patterns encountered thus far (Figs. 13–18). A feature of interest in relation to these band patterns is the frequent occurrence of transitions from one type of packing to another. Figure 18 clearly shows such a transition from the 1800-A type to the 145-A spacing, Fig. 17 a rhythmic transition from the 145-A spacing to a *ca* 70-A spacing and vice versa.

X-RAY DIFFRACTION AND ROTATORY DISPERSION

All of the proteins considered up to this point, with the exception of actin, give wide-angle x-ray diffraction patterns in which the prominent feature is the 5.1-A meridional reflection, indicating that at least a significant proportion of the polypeptide chains possess the α-helical configuration observed by Pauling and Corey.[5,6] This is true also of the meromyosins,[11] LMM giving a good pattern, with HMM being more variable since it is rather easily denatured. However, the x-ray diffraction method does not lend itself to estimation of the noncrystalline but helical parts of the molecule. The helix content of proteins in aqueous solution can best be estimated at the present time by rotatory-dispersion measurements, and the equation by Moffitt[56] describing the rotatory dispersion of the α-helix can be used for this purpose with the muscle proteins, subject to certain theoretical limitations, and on the assumption that the

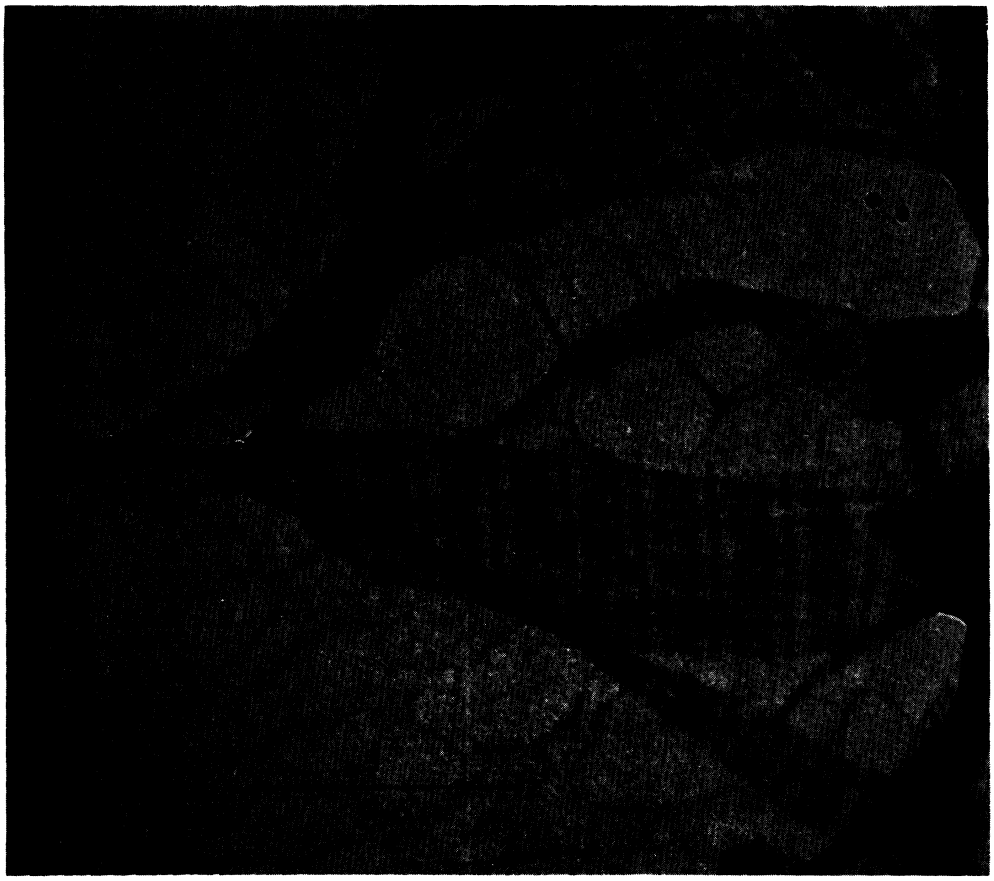

FIG. 14. Fibrils reconstituted from an acid solution of paramyosin showing an axial repeat period of about 1400 A. Note that the band structure within each repeat period is arranged symmetrically. This is interpreted as arising from an antiparallel packing of rod-like molecules about 1400 A long [from A. J. Hodge, Proc. Natl. Acad. Sci. U. S. 38, 850 (1952)]. ×70 000.

entire helical content is in the right-handed α-helical configuration. Table III, from the work of Cohen and Szent-Györgyi,[11] shows the estimated α-helix content in aqueous solutions of the muscle proteins and fragments under discussion. It can be seen from this table also that the extent of the helical regions seems to be dependent upon the proline content. The proline contents of the highly helical muscle components (LMM fr. I, tropomyosin, and paramyosin) are very low, while those of myosin and HMM (which exhibit the lowest helix content) are correspondingly high. On a statistical basis, the data indicate that each proline residue prevents about 20 other residues from participating in α-helix formation.

TABLE III. Helix content and proline and cystine concentration of muscle proteins [from C. Cohen and A. G. Szent-Györgyi, paper read at International Biochemistry Congress, Vienna (1958)].

Protein	Wt. % helix	Equ. cystine in 10^5 g	Wt. % proline	No. of nonhelical residues per proline residue
LMM fr. 1	100	0	0.22	
Tropomyosin	94	1.65	0.35	15
Paramyosin	91	0	0.21	36
LMM	74	0.65	0.97	23
Myosin	56	0.6	2.08	18
HMM	45	1.2	2.87	16

LOCALIZATION OF THE MUSCLE PROTEINS IN THE MYOFIBRIL

The commonly accepted distribution of actin, myosin, and tropomyosin in striated muscle as deduced from extraction experiments of a more or less selective nature is shown in Fig. 19, reproduced from a review by Perry.[57] However, there are indications that the picture is not quite so simple, especially in relation to the distribution of myosin and to the changes in the density distribution within the sarcomere accompanying shortening. According to the concept of Huxley and Hanson,[9] the actin and myosin are in the form of two separate sets of interdigitating filaments. Shortening is accomplished not by contraction of the filaments themselves, but rather by a mutual sliding action. As the *I* bands disappear, the ends of the myosin filaments pile up against the *Z* band to produce the well-known contraction bands (C_z bands). However, there are many indications in the literature (e.g., Hodge[58]) which suggest that at least some of the material contributing to *A*-band density in the relaxed myofibril is capable of actual migration to the *Z* bands (and possibly to the *M* bands)

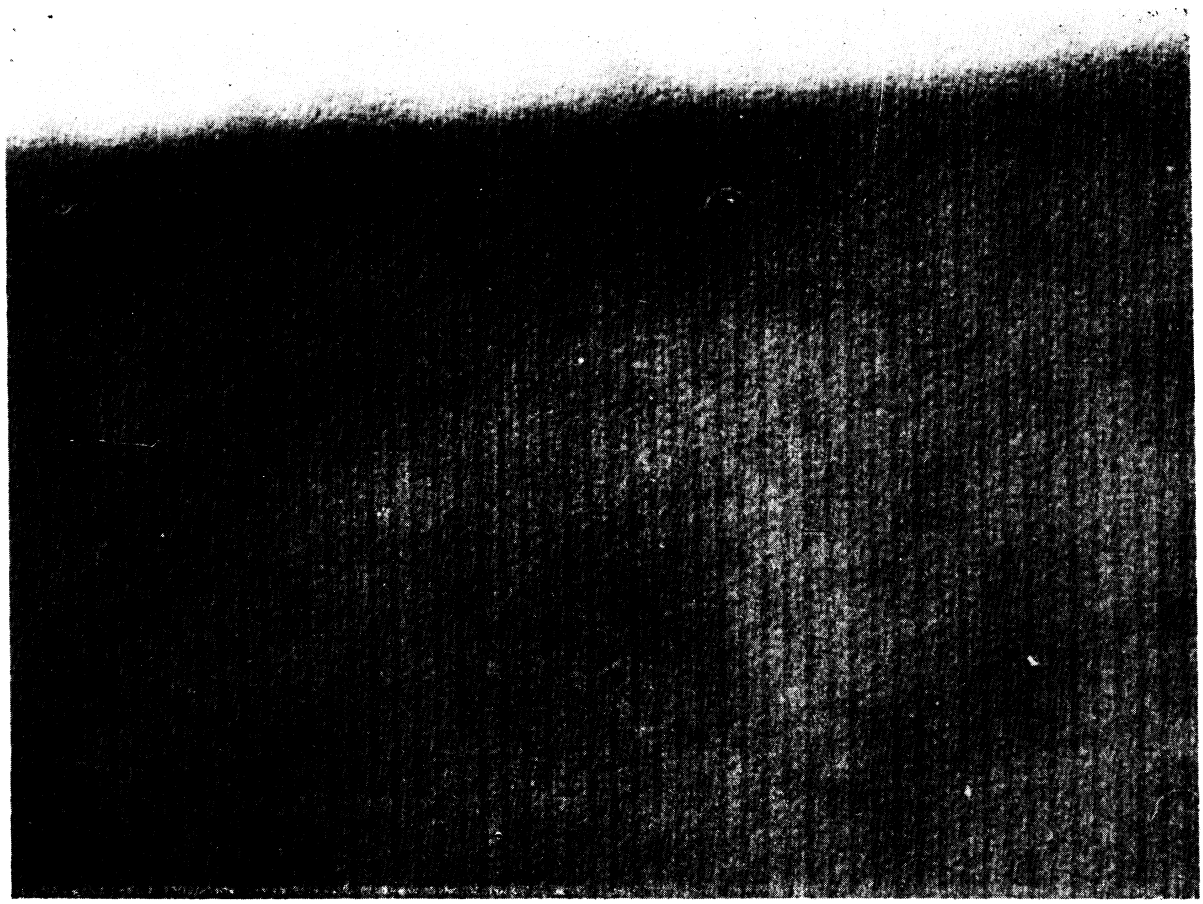

FIG. 15. Electron micrograph of a large flat paramyosin "crystal" obtained by reducing the ionic strength of an approximately neutral paramyosin solution, stained with PTA. The axial period is about 145 A. ×190 000.

independently of any sliding of formed myosin elements. Possibly, such substances act as moderators, activators, or inhibitors in controlling the orderly interaction of the actin, myosin, and other macromolecular components.

There is evidence to indicate that the myosin content of the *A* band is not homogeneous with respect to its extractibility. Thus, on extraction with Guba-Straub-ATP solution, a band of appreciable density remains on both sides of the *M* band[59] (Fig. 20), which can be removed only by further extraction with a pyrophosphate solution.[60] Furthermore, it has been shown that appreciable amounts of components other than myosin are extracted from the *A* band by these procedures.[61] The distribution of birefringence in the sarcomere as found by Inoué and Szent-Györgyi[62] using high-resolution polarization-optical methods coupled with extraction and replating experiments is also at variance with the simple distribution of actin and myosin as postulated by Huxley and Hanson.[63]

The polarization-optical results are more in harmony with the very interesting results of Holtzer and Marshall[64] using the technique of "fluorescent antibody analysis" to determine the localization of the various muscle components. The distributions of actin and myosin found with this technique are essentially in accord with that shown in Fig. 19. However, when using antibodies specific for LMM and HMM, the very curious result emerges that these two components appear to be localized independently of each other within the $A+H$ region of the sarcomere. The LMM appears to be located preferentially in the *A* bands proper, the HMM in a complementary pattern consisting of two bands, one on each side of and adjacent to the *M* band. More recently, Holtzer and Szent-Györgyi[65] have confirmed these observations and demonstrated that there is no physical hindrance to penetration of the antibody into the myofibril, by carrying out experiments involving extraction with KI solutions. In the control, this treatment removes most of the protein of the myofibril. However, pretreatment of a myofibril with a specific antibody prevents the extraction of the particular protein under consideration. The independent but somewhat overlapping distributions found for LMM and HMM by the above methods have also been confirmed by Inoué and Szent-Györgyi,[62] who first selectively extracted for myosin and then exposed the

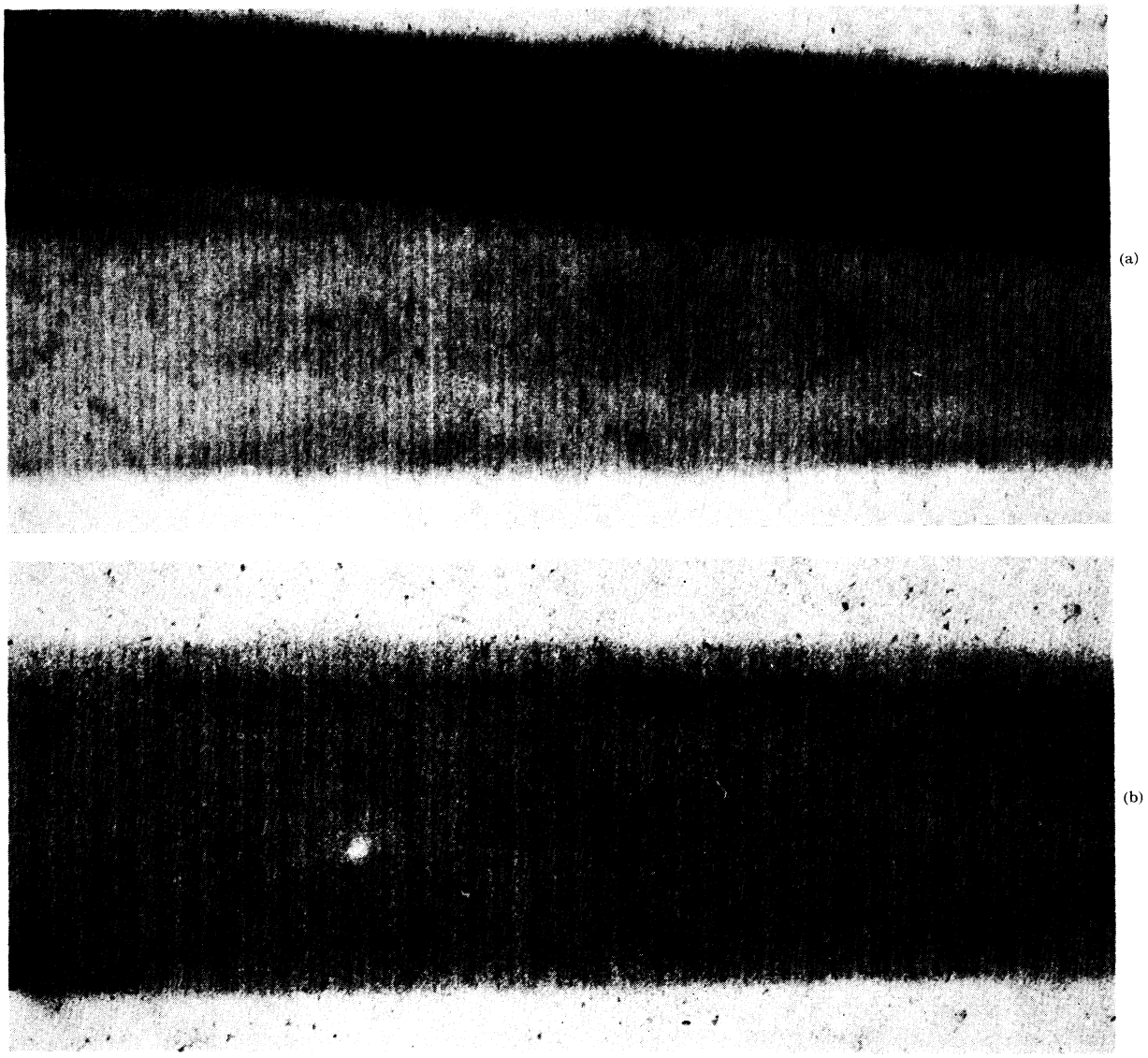

FIG. 16. Two fibrils from the same preparation as Fig. 15. The upper one (a) shows a simple period of about 145 A with a superposed apparently isomorphous structure having a repeat of 725 A, the lower one (b) shows a more complex pattern in which the fundamental repeat period is 5×145 A=725 A. Stained with PTA. Similar patterns have been observed by Hanson *et al.*[56] ×125 000.

myofibrils to LMM and HMM solutions before polarization-optical examination. They found birefringent bands appearing in positions consistent with the fluorescent-antibody results. This apparent independence of at least some of the LMM and HMM within the myofibril recalls the observations of metabolic independence mentioned earlier, and raises the question of whether or not some kind of interaction or reaction of LMM with HMM is involved in the process of contraction.

PERSPECTIVES

The sliding filament model of muscle is based, to a considerable extent, on the observation in the electron microscope of two sets of filaments in the *A* bands, identified by Huxley and Hanson as actin and myosin. However, these filaments have been observed, of necessity, only in thin sections of muscle subjected to the procedures involved in fixation, dehydration, and embedding, so that the original state in the living, resting muscle fiber is a matter of inference. The danger of extrapolation from such observations is illustrated by comparing the low-angle x-ray diffraction data with the results of electron microscopy for muscle fixed (a) in the fresh state and (b) after glycerination. Living muscle gives a low-angle equatorial x-ray diffraction pattern consisting of two reflections corresponding to rod-like elements about 450 A apart in a hexagonal array,[67]

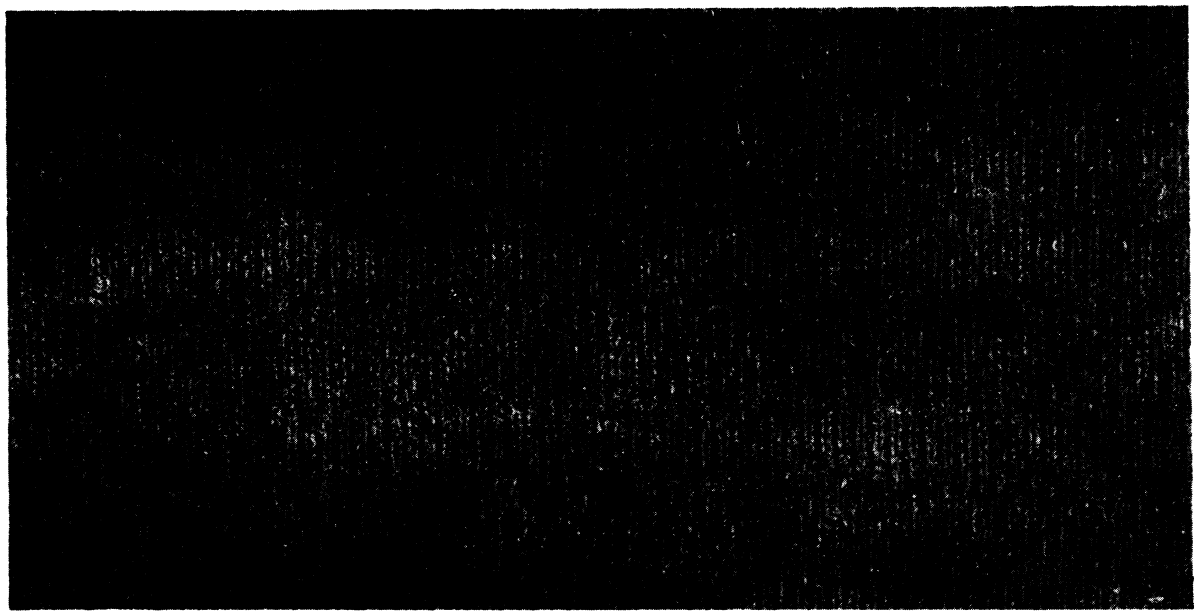

FIG. 17. Paramyosin crystals from the same preparation as Fig. 15, showing a rhythmic transition from a 145-A repeat structure to a *ca* 70-A repeat structure and vice versa. The author is grateful to J. L. Farrant for his suggestion that this pattern could result from the superposition (with small angular displacement of fiber axes) of two paramyosin crystals with 145-A spacings similar to that shown in Fig. 15. ×160 000.

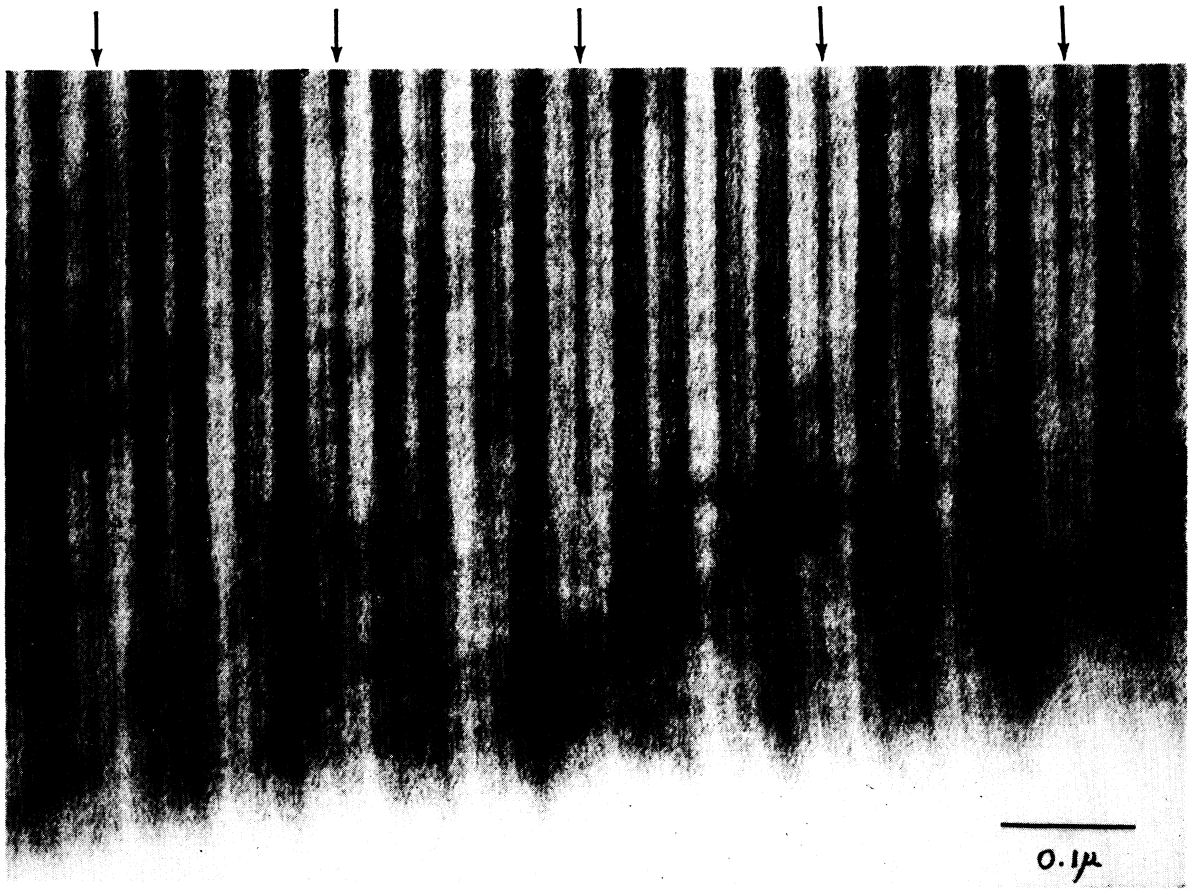

FIG. 18. Paramyosin "crystal" obtained by dilution of a solution of paramyosin in neutral high ionic strength phosphate buffer and stained with PTA, showing a complex band pattern of the type described by Locker and Schmitt[55] with an axial repeat period of about 1800 A (indicated by arrows at top), and a region of transition from the 1800-A repeat structure to one in which the 145-A spacing is accentuated. ×180 000.

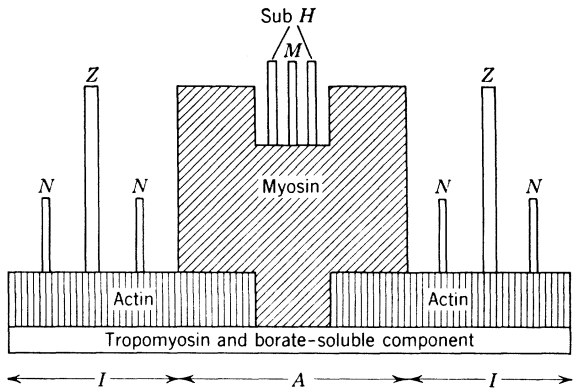

FIG. 19. Diagram illustrating the distribution of the protein components of the myofibril within the sarcomere. The height of the shaded areas represents the protein density at any point along the myofibril axis [from S. V. Perry, Physiol. Rev. **36**, 1 (1956)].

with a strong first order and with a relatively weak second order. If the ATP is removed from the muscle (i.e., in glycerinated muscle or muscle in rigor), a striking reversal of the intensities of these two reflections takes place, the second order becoming very strong.[67] This indicates that, in the absence of ATP, a region of high electron density is present at a position intermediate between the primary rod-like elements, while, when ATP is present, the electron density between these elements is relatively uniform. The electron microscope results are at variance with this, for the two sets of filaments can be observed equally well in muscle fixed in the fresh state or after glycerination.[37] It thus is necessary to conclude that one (or more) of the processes involved in fixation, dehydration, and embedding causes the muscle to go into a rigor-like state, and consequently, that the density distributions observed in electron micrographs do not correspond to those present in the living, resting muscle fiber. The results clearly demonstrate the need for a thorough correlation by x-ray diffraction of the procedures used in the preparation of specimens for the electron-microscopic examination of muscle.

In the sliding-filament model, the two sets of filaments are postulated to remain at constant length on the basis of evidence derived largely from observations of *A*-band lengths during shortening. Thus, this mechanism does not require configurational changes of the protein components, neither at the α-helix nor at the macromolecular level. No direct evidence on this point has been reported. However, Huxley[67] found no change in the axial spacing measured from the low-angle x-ray diffraction pattern on passive stretching of the muscle, a result which has been interpreted as possibly indicating that no change would accompany shortening. On the other hand, some as yet inconclusive evidence from electron-microscopical observations[32,37] suggests that there is a correlation of the axial period both with the

FIG. 20. Electron micrographs of thin sections through (a) intact glycerinated fibril and (b) glycerinated fibril after extraction with Guba-Straub-ATP solution [from J. Hanson and H. E. Huxley, Nature **172**, 530 (1953)].

sarcomere length and with the type of band pattern observed during shortening. Such a variation in the axial period would strongly imply the occurrence of configurational changes during shortening. It is clear that further evidence of a more direct nature is urgently needed in order to settle this matter.

As has been seen, the application of analytical and degradative methods has proved to be of great importance in solving many of the problems associated with muscle structure and function. However, it would seem that the time is ripe for an approach to muscle which considers the whole machine as a complex mixed macromolecular "crystal," capable of changes in physical state in order to convert available chemical energy into mechanical work. Any completely adequate theory of contraction must be able to take into account the interactions between the many and varied components of the myofibril and other contractile units.

BIBLIOGRAPHY

[1] W. A. Engelhardt and M. N. Ljubimowa, Nature **144**, 668 (1939).

[2] Albert Szent-Györgyi, Acta Physiol. Scand. **9**, Suppl., 25 (1945).

[3] Albert Szent-Györgyi, *Chemistry of Muscular Contraction* (Academic Press, Inc., New York, 1951), second edition.

[4] B. B. Marsh, Nature **167**, 1065 (1951).

[5] L. Pauling and R. B. Corey, Nature **171**, 59 (1953).

[6] L. Pauling and R. B. Corey, Proc. Roy. Soc. (London) **B141**, 21 (1953).
[7] F. H. C. Crick, Acta Cryst. **6**, 689 (1953).
[8] R. S. Bear, J. Am. Chem. Soc. **67**, 1625 (1945).
[9] H. E. Huxley and J. Hanson, Symp. Soc. Exptl. Biol. **9**, 228 (1955).
[10] F. B. Straub, Studies Inst. Med. Chem. Univ. Szeged **2**, 3 (1942).
[11] C. Cohen and A. G. Szent-Györgyi. Paper read at International Biochemistry Congress, Vienna (1958).
[12] W. F. H. M. Mommaerts, J. Biol. Chem. **198**, 445 (1952).
[13] T. C. Tsao, Biochim. et Biophys. Acta **11**, 227 (1953).
[14] K. Laki and W. R. Carroll, Nature **175**, 389 (1955).
[15] A. Holtzer and S. Lowey, J. Am. Chem. Soc. **78**, 5954 (1956).
[16] W. F. H. M. Mommaerts and B. B. Aldrich, Science **126**, 1294 (1957).
[17] J. Gergely and H. Kohler, Conference on the Chemistry of Muscular Contraction, Tokyo (1957).
[18] A. G. Szent-Györgyi, Arch. Biochem. Biophys. **42**, 305 (1953).
[19] J. Gergely, H. Kohler, W. Ritschard, and L. Varga, Abstract of Meeting of the Biophysical Society, Boston (1958), p. 46.
[20] T. C. Tsao, K. Bailey, and G. S. Adair, Biochem. J. **49**, 27 (1951).
[21] C. M. Kay, Biochim. et Biophys. Acta **27**, 469 (1958).
[22] W. T. Astbury and L. C. Spark, Biochim. et Biophys. Acta **1**, 388 (1947).
[23] W. T. Astbury, Nature **160**, 388 (1947).
[24] C. C. Selby and R. S. Bear, J. Biophys. Biochem. Cytol. **2**, 71 (1956).
[25] J. Gergely, J. Biol. Chem. **200**, 543 (1953).
[26] E. Mihályi, J. Biol. Chem. **201**, 197 (1953).
[27] E. Mihályi and A. G. Szent-Györgyi, J. Biol. Chem. **201**, 211 (1953).
[28] J. Gergely, M. A. Gouvea, and D. Karibian, J. Biol. Chem. **212**, 165 (1955).
[29] W. R. Middlebrook, Abstract of Meeting of Biophysical Society, Boston (1958), p. 46.
[30] D. E. Philpott and A. G. Szent-Györgyi, Biochim. et Biophys. Acta **15**, 165 (1954).
[31] C. E. Hall, M. A. Jakus, and F. O. Schmitt, Biol. Bull. **90**, 32 (1946).
[32] M. H. Draper and A. J. Hodge, Australian J. Exptl. Biol. Med. Soc. **27**, 465 (1949).
[33] M. H. Draper and A. J. Hodge, Nature **163**, 576 (1949).
[34] M. H. Draper and A. J. Hodge, Australian J. Exptl. Biol. Med. Soc. **28**, 549 (1950).
[35] S. Leisegang, *Proceedings of the Third International Conference on Electron Microscopy*, London, July 1954 (Royal Microscopical Society, London, 1956), p. 176.
[36] A. J. Hodge, H. E. Huxley, and D. Spiro, J. Exptl. Med. **99**, 201 (1954).
[37] A. J. Hodge, J. Biophys. Biochem. Cytol. **2**, Suppl., 131 (1956).
[38] S. F. Velick, Biochim. et Biophys. Acta. **20**, 228 (1956).
[39] G. Schapira, G. Broun, J. C. Dreyfus, and J. Kruh, Compt. rend. soc. biol. **150**, 944 (1956).
[40] K. Bailey, Biochem. J. **43**, 271 (1948).
[41] A. J. Hodge, A. G. Szent-Györgyi, and C. Cohen (paper in preparation).
[42] M. A. Jakus, J. Biophys. Biochem. Cytol. **2**, Suppl., 243 (1956).
[43] C. Rouiller and E. Fauré-Fremiet, J. Ultrastructure Research **1**, 1 (1957).
[44] C. E. Hall, M. A. Jakus, and F. O. Schmitt, J. Appl. Phys. **16**, 459 (1945).
[45] F. O. Schmitt, R. S. Bear, C. E. Hall, and M. A. Jakus, Ann. New York Acad. Sci. **47**, 799 (1947).
[46] R. S. Bear and C. C. Selby, J. Biophys. Biochem. Cytol. **2**, 55 (1956).
[47] G. F. Elliott, J. Hanson, and J. Lowy, Nature **180**, 1291 (1957).
[48] A. J. Hodge, "Studies on paramyosin; the *in vitro* reconstitution and transformation of periodic structure," Ph.D. thesis, Massachusetts Institute of Technology (1952).
[49] A. J. Hodge, Proc. Natl. Acad. Sci. U. S. **38**, 850 (1952).
[50] F. O. Schmitt, J. Gross, and J. H. Highberger, Proc. Natl. Acad. Sci. U. S. **39**, 459 (1953).
[51] K. Bailey, Publ. Staz. Zool. Napoli **29**, 96 (1956).
[52] K. Bailey, Biochim. et Biophys. Acta **24**, 612 (1957).
[53] D. R. Kominz, F. Saad, and K. Laki, Nature **179**, 206 (1957).
[54] A. G. Szent-Györgyi (personal communication, 1958).
[55] R. H. Locker and F. O. Schmitt, J. Biophys. Biochem. Cytol. **3**, 889 (1957).
[56] W. Moffitt, J. Chem. Phys. **25**, 467 (1956).
[57] S. V. Perry, Physiol. Rev. **36**, 1 (1956).
[58] A. J. Hodge, J. Biophys. Biochem. Cytol. **1**, 361 (1955).
[59] J. Hanson and H. E. Huxley, Nature **172**, 530 (1953).
[60] H. E. Huxley and J. Hanson, Nature **173**, 973 (1954).
[61] A. G. Szent-Györgyi, D. Mazia, and Albert Szent-Györgyi, Biochim. et Biophys. Acta **16**, 339 (1955).
[62] S. Inoué and A. G. Szent-Györgyi (unpublished data).
[63] H. E. Huxley and J. Hanson, Biochim. et Biophys. Acta **23**, 229 (1957).
[64] H. Holtzer and J. M. Marshall, results presented at the Meeting of the Biophysical Society, Boston (1958).
[65] H. Holtzer and A. G. Szent-Györgyi, (unpublished data).
[66] J. Hanson, J. Lowy, H. E. Huxley, K. Bailey, C. M. Kay, and J. C. Ruegg, Nature **180**, 1134 (1957).
[67] H. E. Huxley, Proc. Roy. Soc. (London) **B141**, 59 (1953).

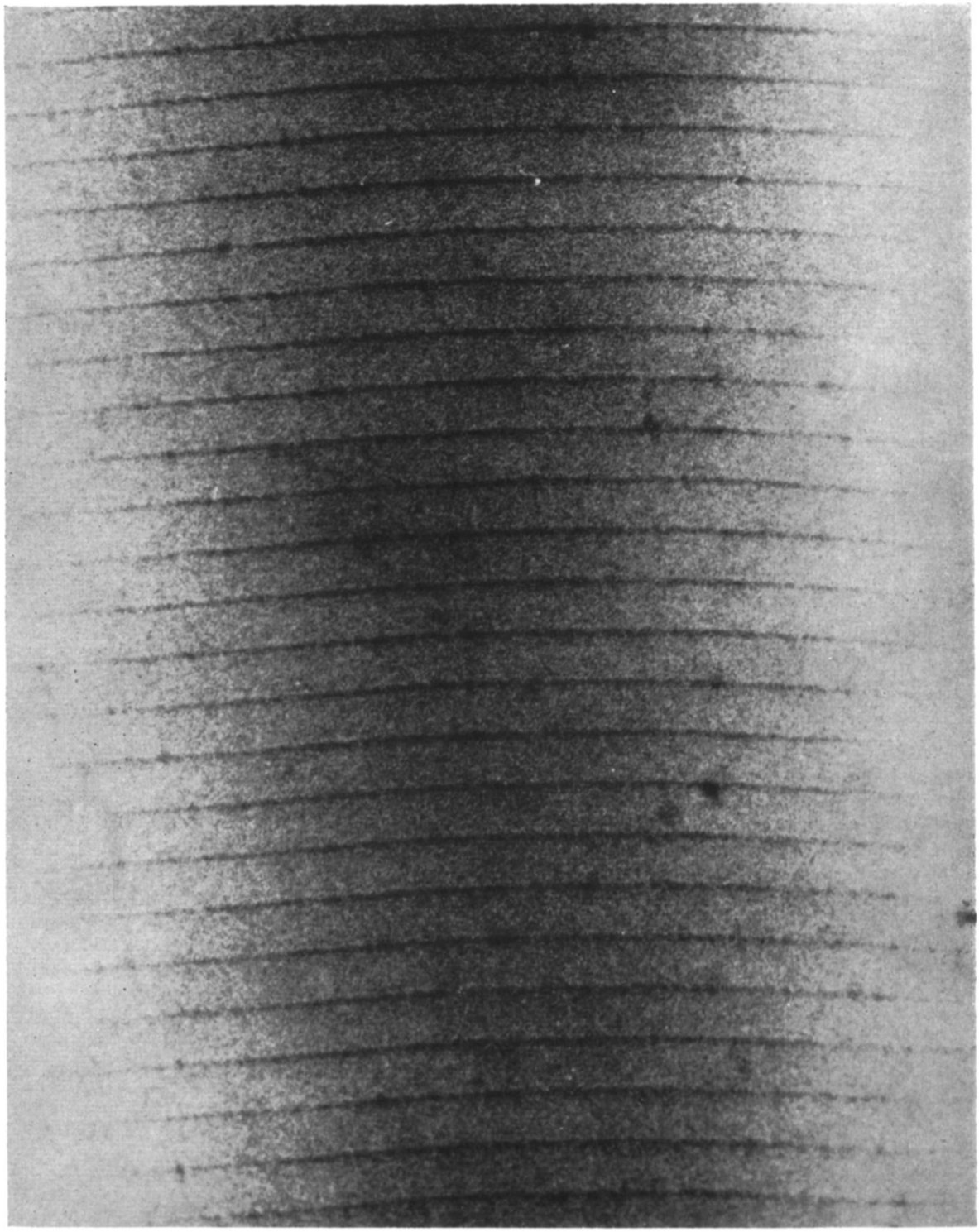

FIG. 1. Crystal of LMM showing the sharp axial banding observed in the electron microscope in the absence of an "electron stain." The very dense transverse striations spaced about 420 A apart are probably owing to binding of salts by the end regions of the LMM molecules [from D. E. Philpott and A. G. Szent-Györgyi, Biochim. et Biophys. Acta **15**, 165 (1954)]). ×200 000.

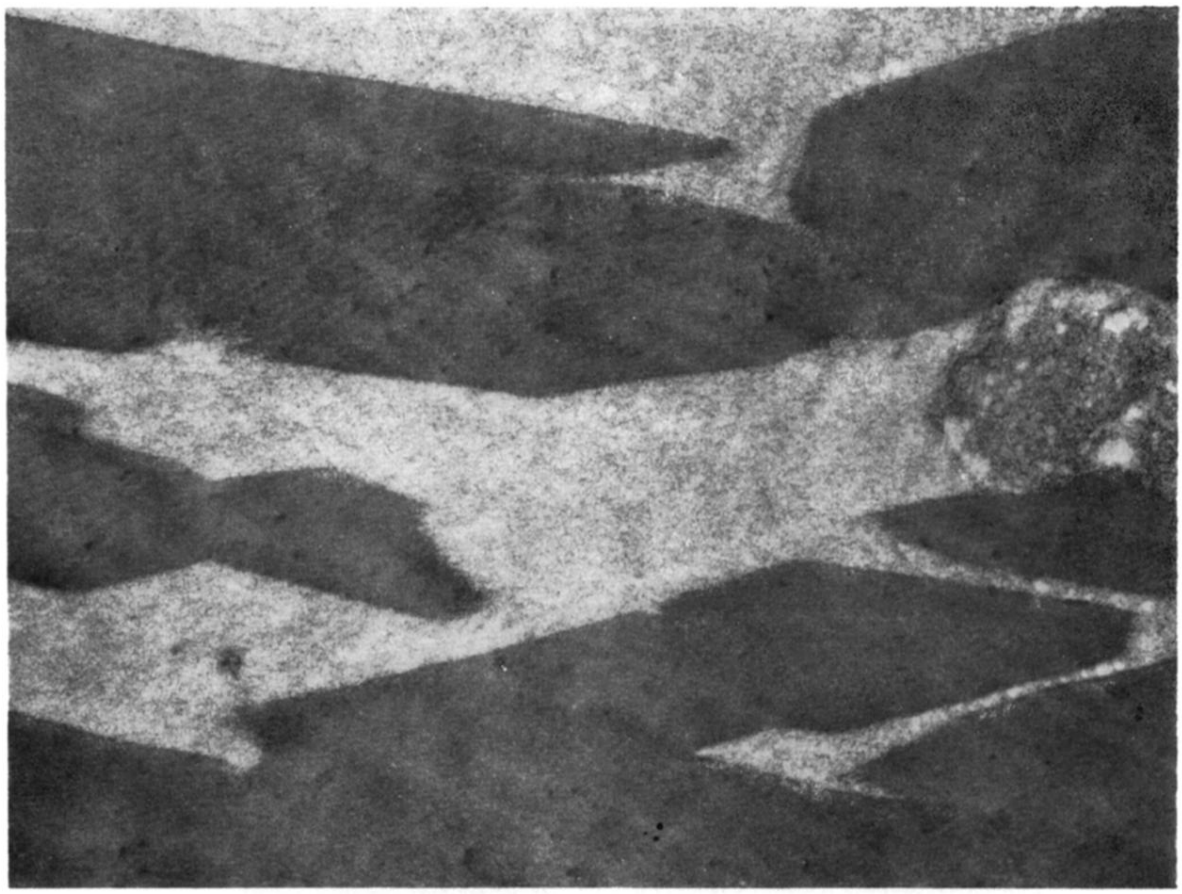

FIG. 10. Electron micrograph of a thin section of rabbit tropomyosin crystals, fixed in buffered, osmium-tetroxide solution, stained with PTA, and embedded in *n*-butyl methacrylate. Note the regular outlines of the crystals and the precise cross-striation corresponding to a spacing of about 200 A. ×20 000.

FIG. 11. Higher magnification view of two tropomyosin crystals in the same preparation as Fig. 10, showing the regular open-network lattice characteristic of these crystals. ×95 000.

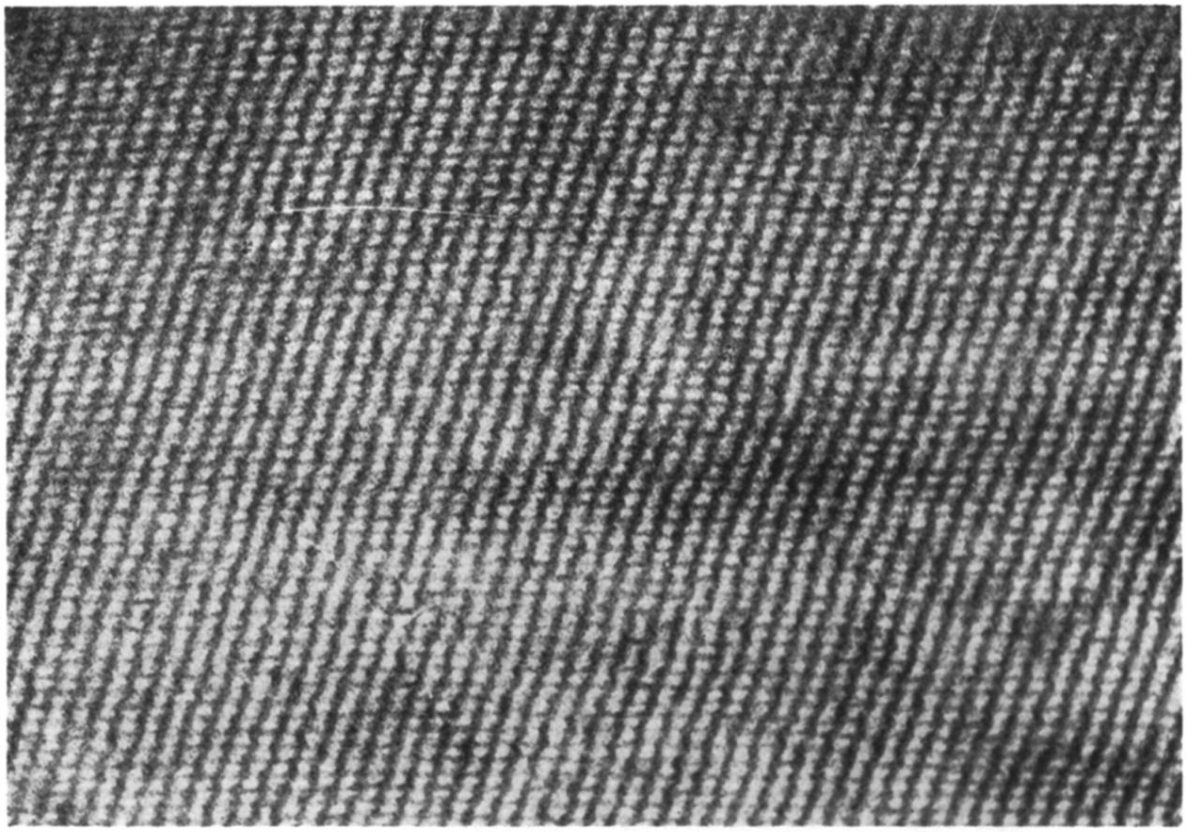

FIG. 12. Thin section of a rabbit tropomyosin crystal showing the characteristic open-network lattice. This type of structure probably accounts for the very high degree of hydration of the tropomyosin crystals. ×190 000.

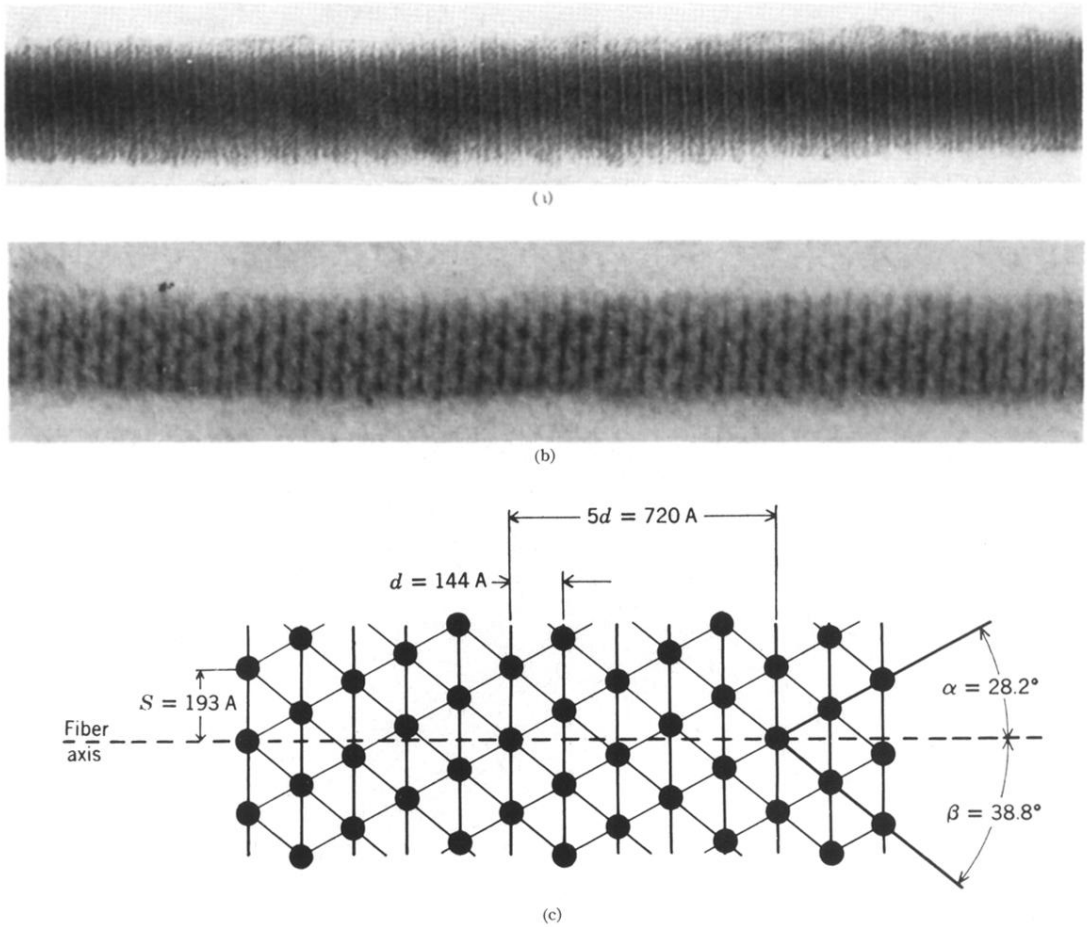

FIG. 13. Native "paramyosin fibrils" isolated from the adductor muscles of *Venus mercenaria* in dilute salt solutions. These usually show (a) a transverse cross-striation with an apparent period of 145 A,[48,49] (b) a pattern consisting of the 145-A cross-striation and a superposed dot pattern of symmetry such that the true repeat period is 5×145 A=725 A [from F. O. Schmitt, R. S. Bear, C. E. Hall, and M. A. Jakus, Ann. N. Y. Acad. Sci. **47**, 799 (1947)]. (c) shows the node pattern derived from electron micrographs. This formal net structure is in good agreement with the results of low-angle x-ray diffraction studies [from C. E. Hall, M. A. Jakus, and F. O. Schmitt, J. Appl. Phys. **16**, 459 (1945)].[8] (a) and (b) ×190 000.

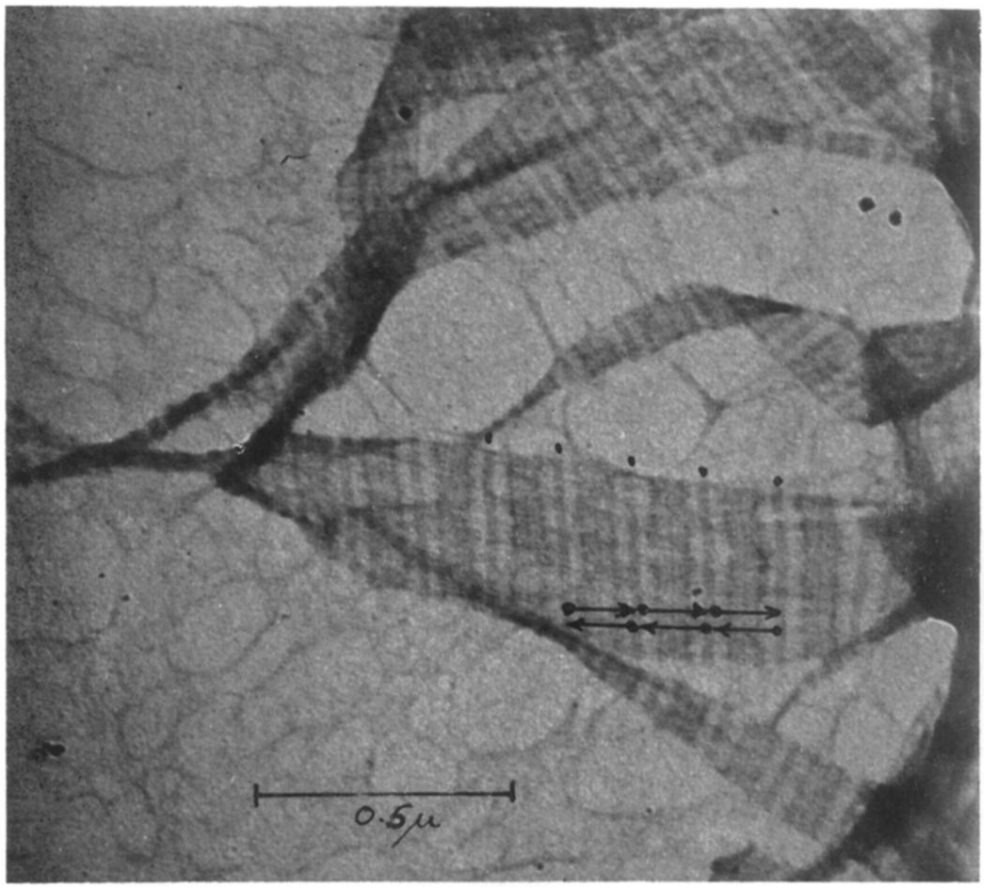

FIG. 14. Fibrils reconstituted from an acid solution of paramyosin showing an axial repeat period of about 1400 A. Note that the band structure within each repeat period is arranged symmetrically. This is interpreted as arising from an antiparallel packing of rod-like molecules about 1400 A long [from A. J. Hodge, Proc. Natl. Acad. Sci. U. S. **38**, 850 (1952)]. ×70 000.

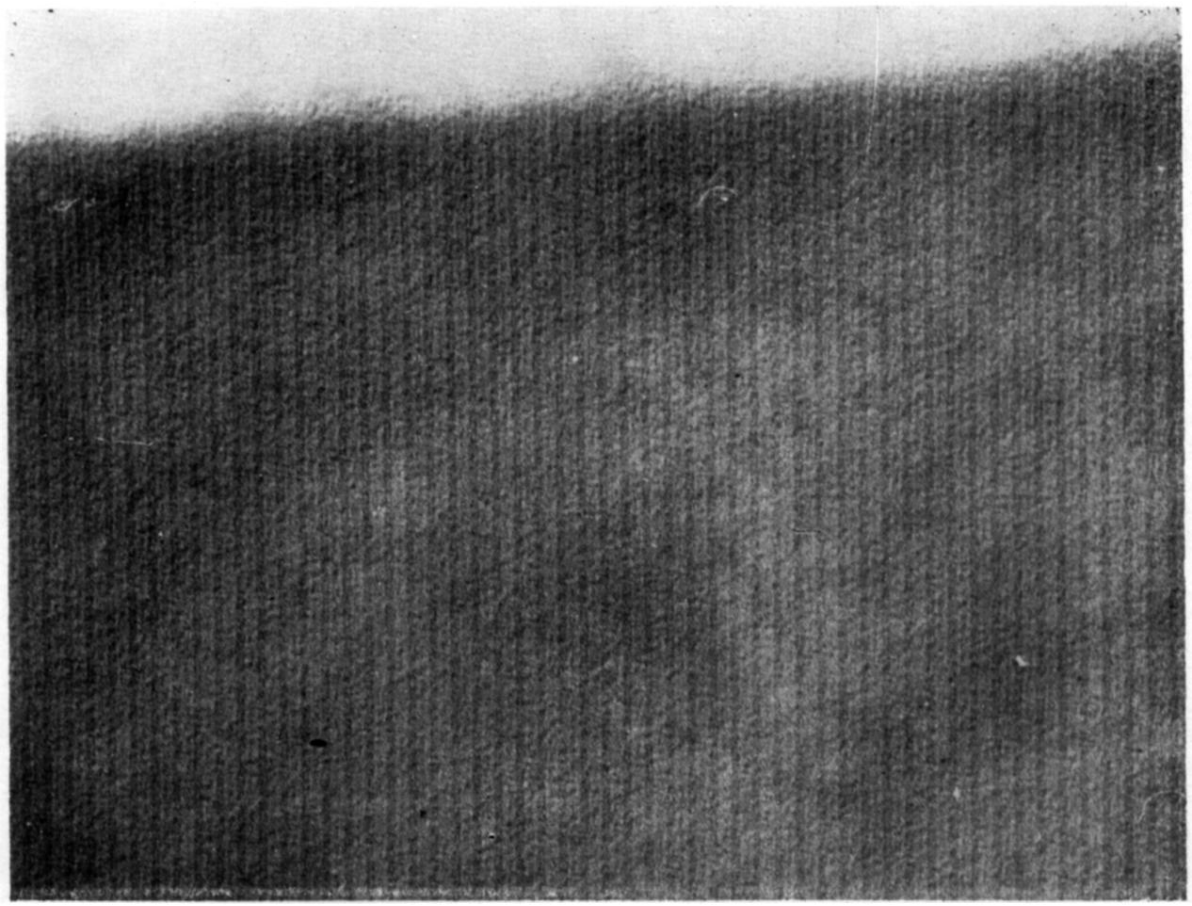

FIG. 15. Electron micrograph of a large flat paramyosin "crystal" obtained by reducing the ionic strength of an approximately neutral paramyosin solution, stained with PTA. The axial period is about 145 A. ×190 000.

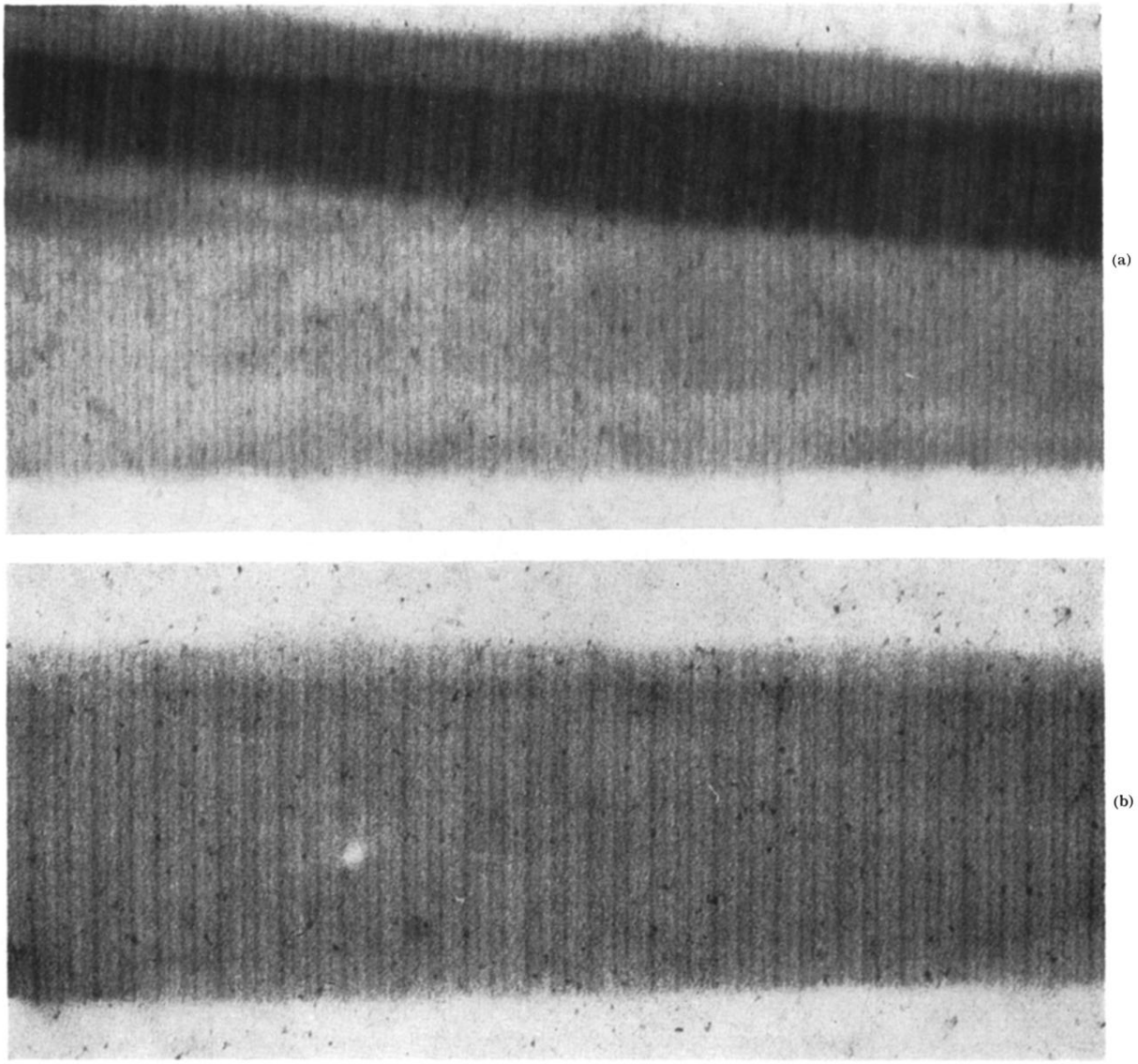

FIG. 16. Two fibrils from the same preparation as Fig. 15. The upper one (a) shows a simple period of about 145 A with a superposed apparently isomorphous structure having a repeat of 725 A, the lower one (b) shows a more complex pattern in which the fundamental repeat period is 5×145 A $= 725$ A. Stained with PTA. Similar patterns have been observed by Hanson *et al.*[56] $\times 125\,000$.

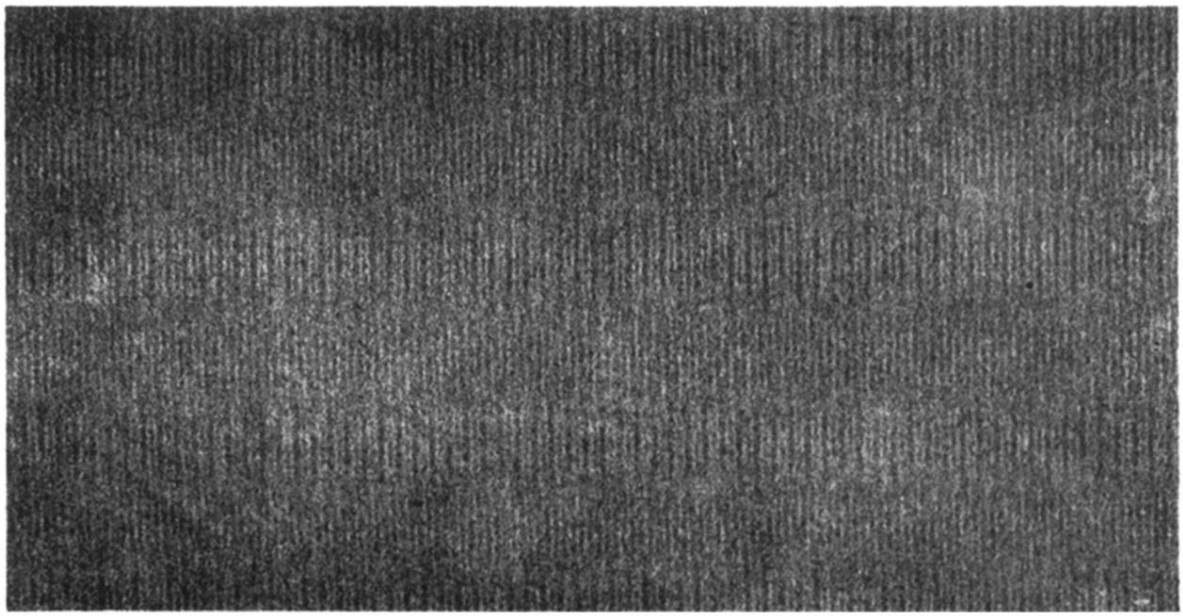

FIG. 17. Paramyosin crystals from the same preparation as Fig. 15, showing a rhythmic transition from a 145-A repeat structure to a *ca* 70-A repeat structure and vice versa. The author is grateful to J. L. Farrant for his suggestion that this pattern could result from the superposition (with small angular displacement of fiber axes) of two paramyosin crystals with 145-A spacings similar to that shown in Fig. 15. ×160 000.

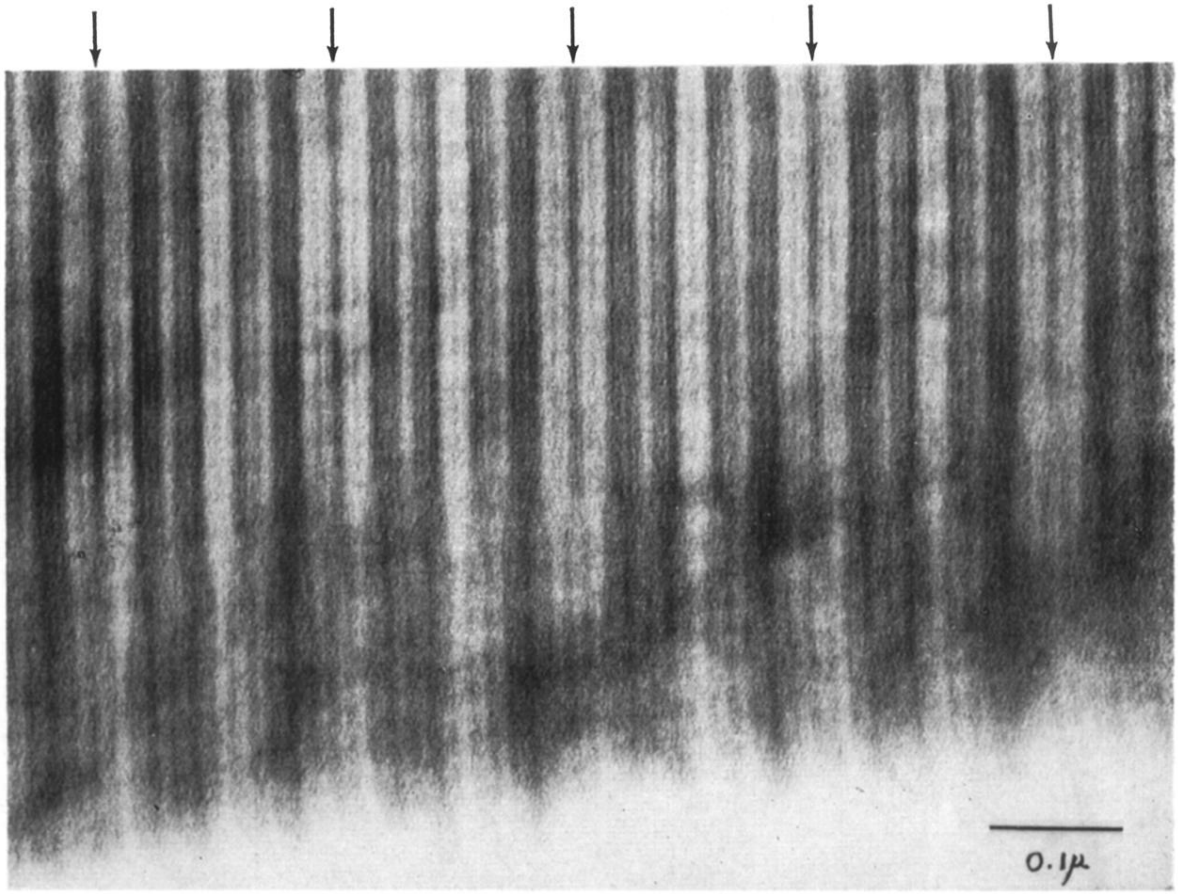

FIG. 18. Paramyosin "crystal" obtained by dilution of a solution of paramyosin in neutral high ionic strength phosphate buffer and stained with PTA, showing a complex band pattern of the type described by Locker and Schmitt[55] with an axial repeat period of about 1800 A (indicated by arrows at top), and a region of transition from the 1800-A repeat structure to one in which the 145-A spacing is accentuated. ×180 000.

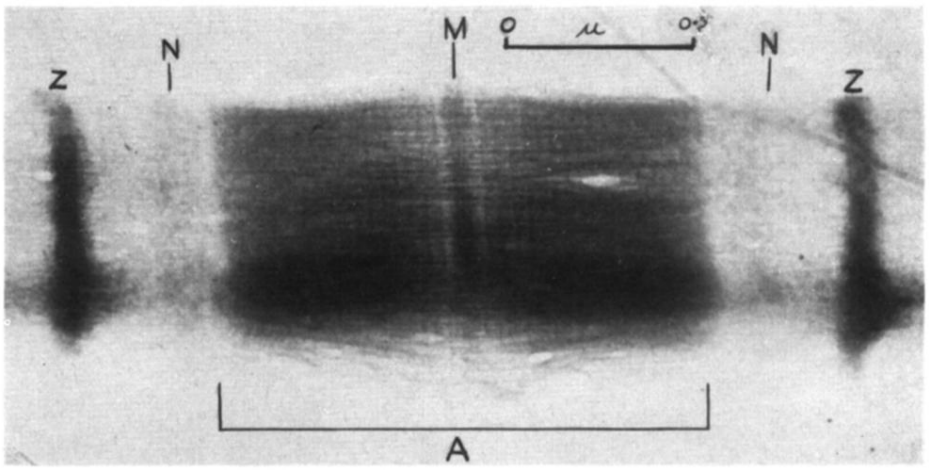

FIG. 2. Electron micrograph of a myofibril isolated from formalin-fixed toad muscle and stained with phosphomolybdic acid, showing the regular 400-A cross-striation in the form of fine, transversely oriented, dense lines [from M. H. Draper and A. J. Hodge, Australian J. Exptl. Biol. Med. Soc. **27**, 465 (1949)]. ×64 000.

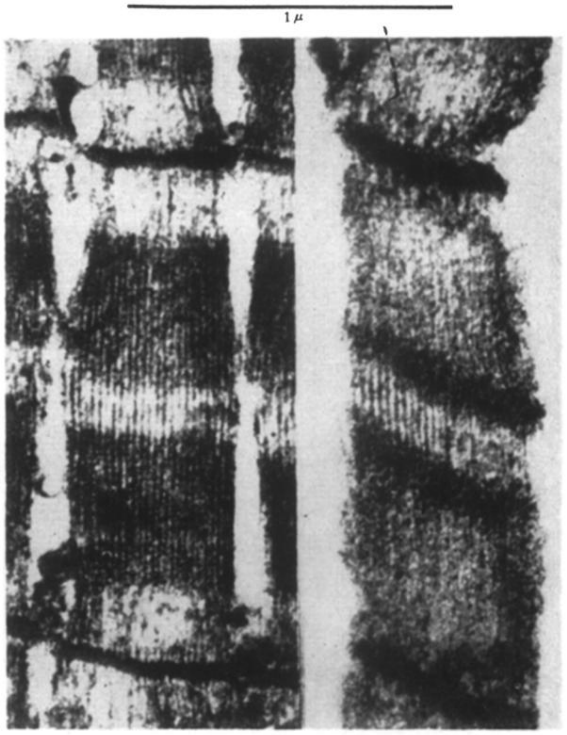

FIG. 20. Electron micrographs of thin sections through (a) intact glycerinated fibril and (b) glycerinated fibril after extraction with Guba-Straub-ATP solution [from J. Hanson and H. E. Huxley, Nature **172**, 530 (1953)].

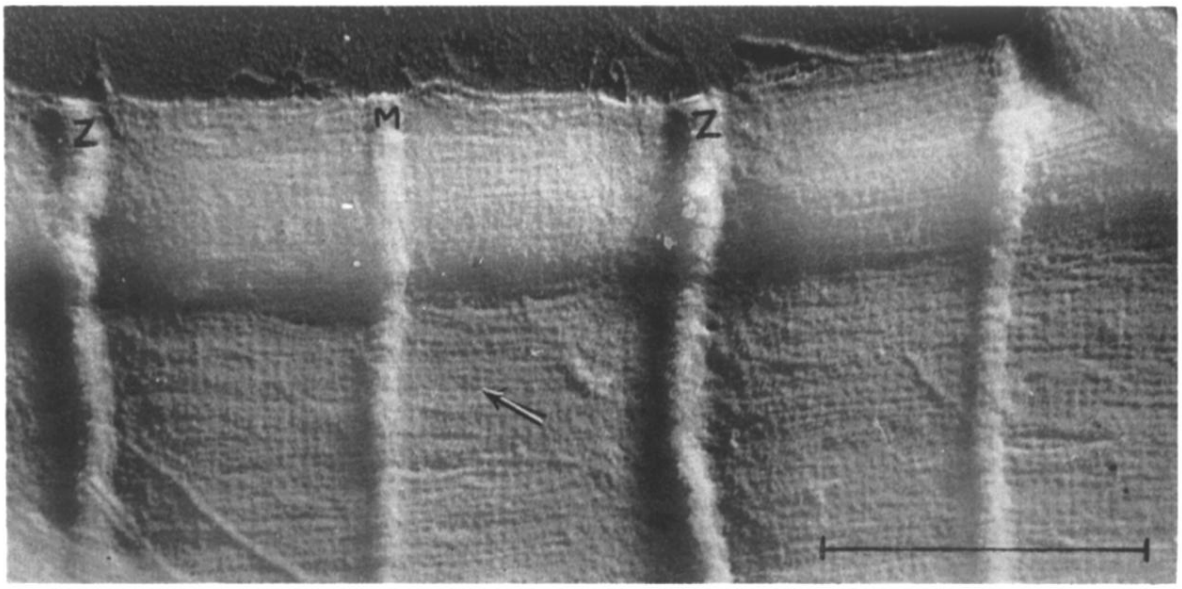

FIG. 3. Semicontracted myofibril of toad muscle, shadowed with platinum, showing the regular axial period apparently associated with the presence of transverse bridges (arrow) [from M. H. Draper and A. J. Hodge, Australian J. Exptl. Biol. Med. Soc. **27**, 465 (1949)]. ×45 000.

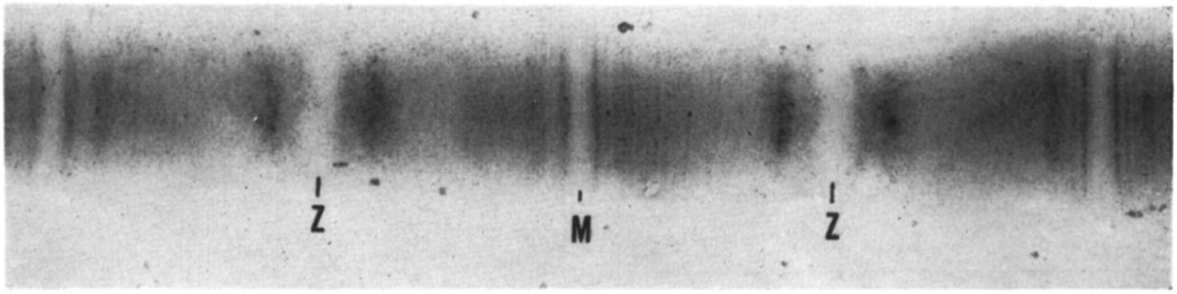

FIG. 4. Formalin-fixed rabbit myofibril after electron-induced microincineration in the electron microscope to illustrate the distribution of the bound mineral residue within the sarcomere in the form of regularly spaced, fine transverse striations spaced about 400 A apart [from M. H. Draper and A. J. Hodge, Nature **163**, 576 (1949)]. ×30 000.

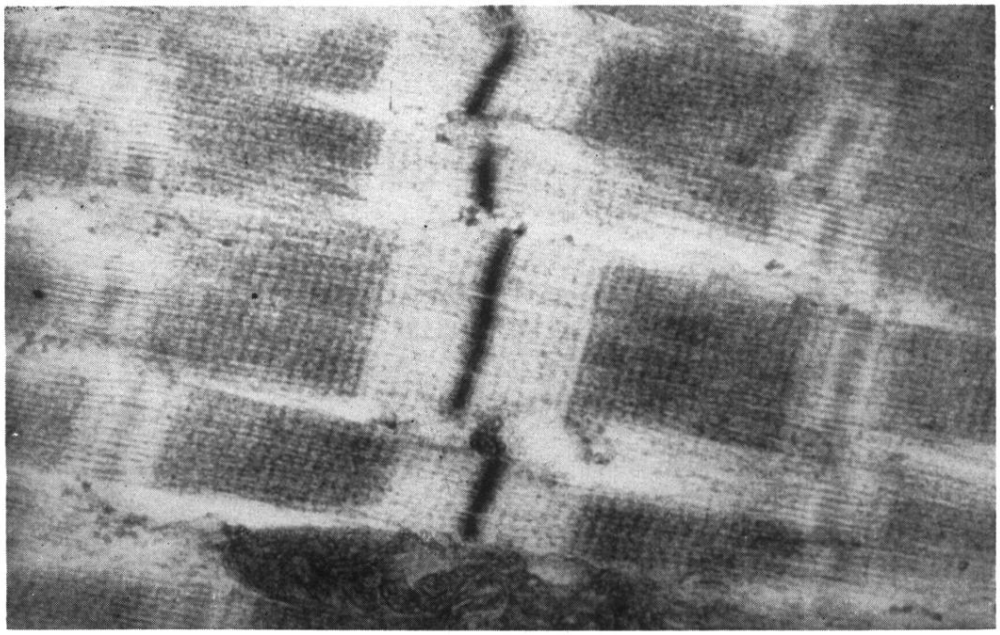

FIG. 5. Thin longitudinal section of rabbit muscle fixed in buffered OsO_4 and stained with PTA, showing the regular cross-striation present in all bands of the sarcomere [from A. J. Hodge, H. E. Huxley, and D. Spiro, J. Exptl. Med. **99**, 201 (1954)]. ×40 000.

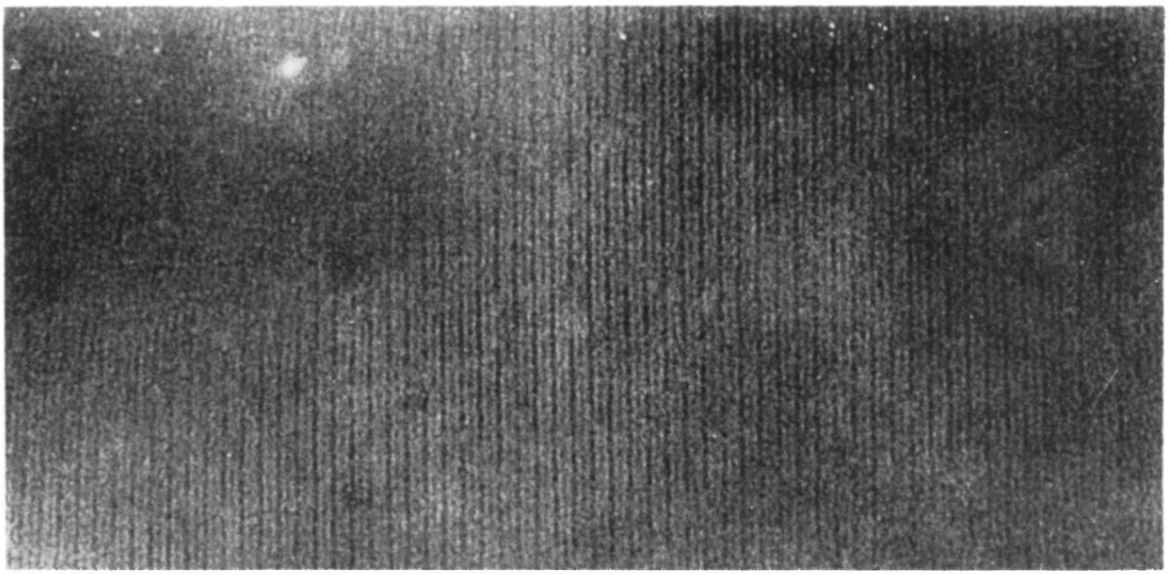

FIG. 6. Electron micrograph of a crystal of rabbit tropomyosin deposited on a supporting film and stained with PTA in *p*H 4.2 phosphate buffer. The main spacing is about 200 A, and a definite intermediate line is present. ×125 000.

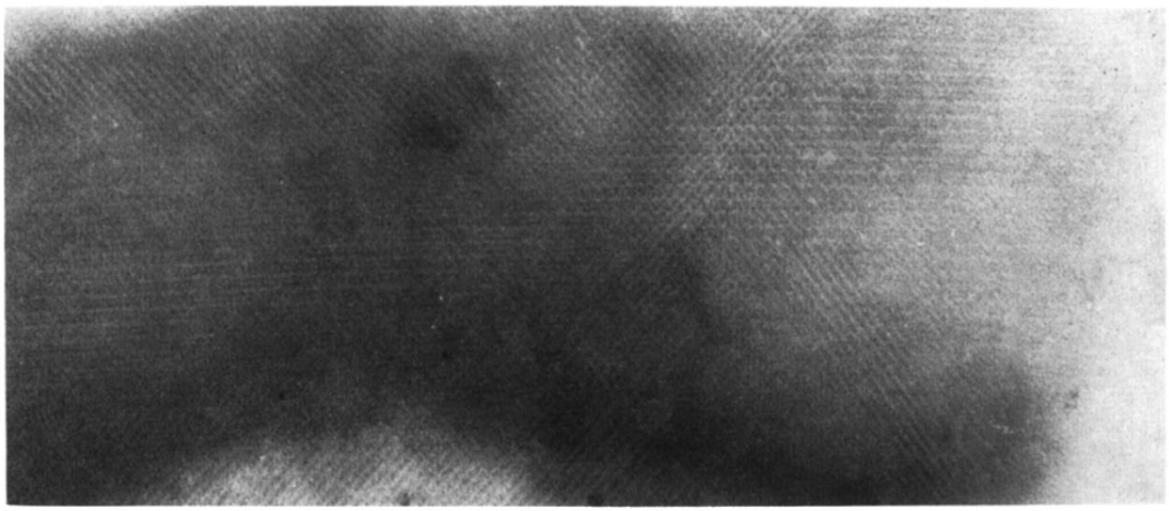

FIG. 7. Crystal of rabbit tropomyosin, stained with PTA, showing the crossed-grid appearance characteristic of many of these crystals in electron microscopic preparations. ×70 000.

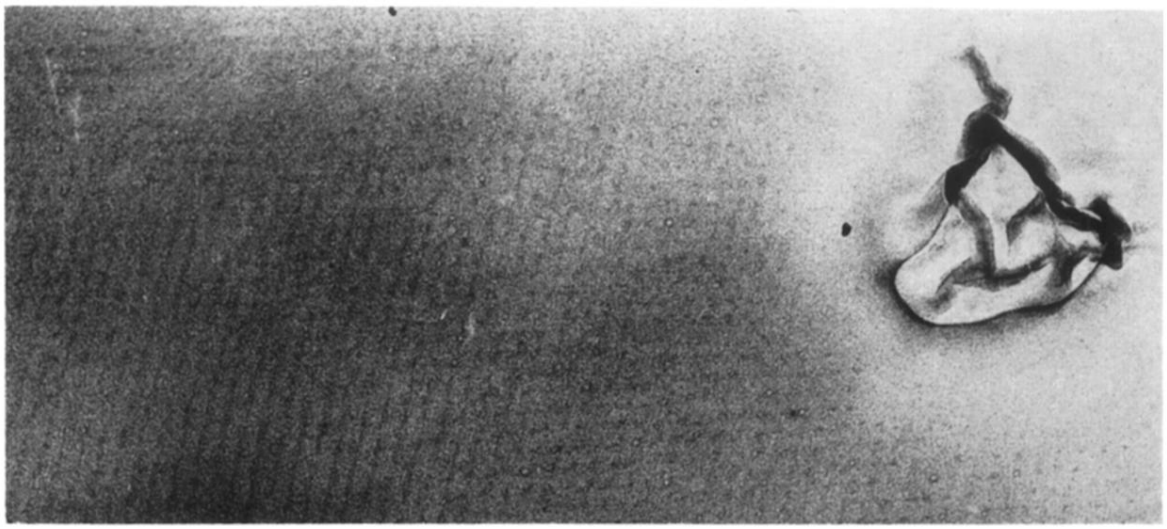

FIG. 8. Rabbit tropomyosin crystal from an ammonium-sulfate suspension mounted on the supporting film without washing or staining. The points and lines of high density probably correspond to regions where salt is accumulated. ×135 000.

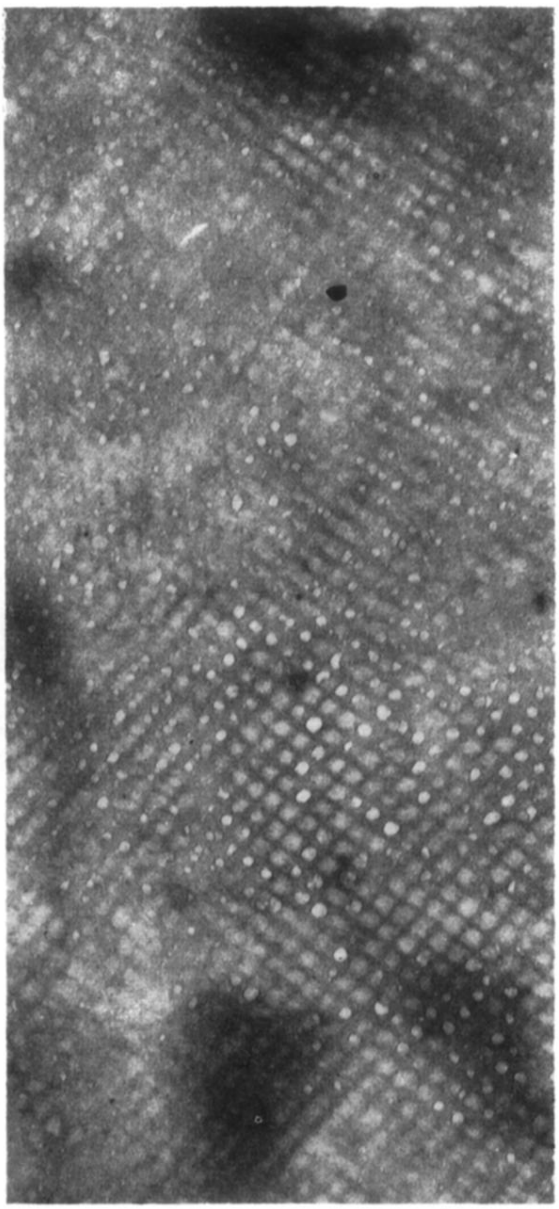

FIG. 9. Open, two-dimensional, net structure observed in a rabbit tropomyosin crystal preparation. The spacing of about 400 A corresponds to the length of the tropomyosin molecule as determined by physicochemical techniques (Table I). ×75 000.