MECHANISM OF SUPERCONTRACTION

IN A STRIATED MUSCLE

GRAHAM HOYLE, JAMES H. McALEAR, and
ALLEN SELVERSTON

From the Department of Biology, University of Oregon, Eugene, Oregon, and the Electron Microscope Laboratory, University of California, Berkeley, California

ABSTRACT

The phenomenon of contraction of a striated muscle down to below 50 per cent rest length has been examined for the scutal depressor of the barnacle *Balanus nubilus* by a combination of phase contrast and electron microscopy. It was found that neurally evoked contraction down to 60 per cent rest length results from the shortening of the I band. At the same time the Z disc changes in structure by an active process which results in spaces opening up within it. Thick filaments can now pass through these spaces from adjacent sarcomeres, interdigitating across the discs. Interdigitation permits repetitive contraction in the living muscle to below 30 per cent rest length. In non-neurally evoked contractions most thick filaments do not find spaces in the Z disc and bend back, giving rise to contraction band artifacts. Expansion of the Z disc can be produced in glycerinated material by the addition of solutions containing a high concentration of ATP.

INTRODUCTION

A satisfactory model of the mechanism of contraction in striated muscle was proposed simultaneously by A. F. Huxley and Niedergerke (1954) and Hanson and H. E. Huxley (1954). In this model, thin filaments of equal length identified with the muscle protein actin attached in a regular array to the Z discs interdigitate with thick filaments identified with myosin present in a regular hexagonal array in the A bands. Contraction occurs by a sliding of the filaments relative to one another. This is brought about by an interaction between the filaments which is probably associated with the action of extensible bridges between them (Davies, 1963). The filaments themselves remain at constant length during the contraction.

With this model as a basis, shortening by up to 50 per cent body length may readily be explained, but further shortening, of which many striated muscles are capable in nature, is impeded when the thick filaments hit the Z discs, where they crumple. The thin filaments are not so impeded, for they are free to interdigitate when they meet in the middle of the sarcomere (Huxley, 1961). We propose to call natural contraction (*i.e.*, as opposed to chemically induced contraction) below 50 per cent rest length[1] *supercontraction*. Although the sliding filament model has received widespread acceptance, some authors have raised objection to it (Sjöstrand, 1962; de Villafranca, 1963; Hodge, 1956). Much of the work on which the hypothesis is based was executed with glycerinated muscle, and it may be argued that the process of glycerination could have altered the basic structure of

[1] Rest length is here defined as the length taken up by isolated fibers attached to a free piece of shell at the basal end, clamped at the tendon end, at the point at which they just fail to kink when the shell rests on a platform.

the material, so that models based on its use cannot represent the natural phenomena. Also, until supercontractions can be accommodated to the theory, its universal applicability remains in doubt.

Recently, striated muscle fibers were found in muscles of the large barnacle *Balanus nubilus* (Hoyle & Smyth, 1963) which are of such giant size that they permit cannulation and are, therefore, of special physiological interest. These fibers fall into the category of fibers that can supercontract, in this case reversibly to $\frac{1}{6}$ rest-length, thereby raising afresh the problem of the detailed mechanism of supercontraction.

By a combination of phase-contrast light microscopy and electron microscopy, we have been able to show that *B. nubilus* giant striated fibers function by a sliding filament mechanism in nature and that supercontraction is possible because the Z discs become perforate and expand during contraction. The thick filaments from neighboring sarcomeres pass through the spaces formed in the Z discs, overlapping as they do so. A preliminary account of these findings has been published (Hoyle and McAlear, 1963).

MATERIALS AND METHODS

Most of the barnacles used were of the species *Balanus nubilus* Darwin, obtained from the Puget Sound region principally by dredging at depths of about 80 feet. A few specimens of the larger barnacle *B. aquilia* from Northern California were also examined; they were obtained by diving. Giant fibers were dissected from scutal depressor muscles which appear to be of homogeneous composition. After dissection in sea water or barnacle Ringer's solution (Hoyle and Smyth, 1963), fibers were subjected to various treatments. Some were first excited to partial or supercontraction by stimulation of the motor nerve; others were stretched to various extents, or examined at body-length. Some were fixed fresh in osmium tetroxide or in permanganate; others were placed in 50 per cent glycerol at 10°C for several weeks prior to examination.

For examination in the phase-contrast microscope, one fiber was cut free from a bundle glycerinated at rest-length. It was then placed in an ATP solution. The routine solution used to excite glycerinated fibers and fibrils to contract was made by dissolving pure sodium adenosine triphosphate (ATP) (Sigma grade-Sigma Chemical Co., St. Louis) in 0.05 M KCl, neutralizing with NaOH, and adjusting to a final concentration of 0.02 M. To this were added magnesium chloride to make 0.001 M concentration and 0.0067 M Tris buffer at pH 7.0. If the fiber contracted

to below 50 per cent rest length, it was considered to be in good condition. About 20 per cent of the glycerinated fibers were rejected. Several fibers were then removed, placed in about 100 ml 0.05 M KCl buffered at pH 7.4, and macerated in a Waring blendor for approximately 30 seconds at high speed. Aliquots of the macerate were centrifuged and the spun-down fibrils were washed twice in 0.05 M KCl at pH 7.0, and transferred to an ice bath. One drop of the suspension was then placed on a slide and covered with a No. 0 coverslip. ATP solutions were washed over the slides by applying drops at one end of the coverslip whilst placing a small square of filter paper at the other end.

The fibrils were observed and photographed with a Leitz Ortholux microscope fitted with a Heine phase-contrast condenser and X 63 dry type objective. Contractions were filmed using a high-pressure mercury burner as light source.

For electron microscopy, fixation was carried out mainly in 1 per cent OsO_4 solution with a phosphate veronal–acetate buffer at pH 7.2 for 1 to 3 hours in an ice bath. Dehydration was accomplished in stages through 100 per cent acetone. At the 70 per cent acetone stage, it was accompanied by staining in 1 per cent uranyl nitrate for 1 to 24 hours. Staining was done with lead hydroxide in some preparations. The muscle was embedded in Epon and examined on unsupported grids. Some of the material was also examined after permanganate or glutaraldehyde fixation. The sections were examined on either an Akashi TRS 50E-1 or a Siemens Elmiskop I.

In addition to muscles prepared in the above manner without prior treatment, muscles were first treated in different ways and then transferred directly to fixative or to cold glycerol and later to fixative. One group was first prepared as nerve-muscle preparations (in the manner described by Hoyle and Smyth, 1963) and subjected to a tetanizing stimulus *via* the nerve. This operation was done in air; at maximum contraction under a light load the fibers were quickly covered with ice-cold glycerol. They were then cooled to $-20°C$ and maintained at this temperature for 6 weeks. Others were stretched, whilst monitoring the resting tension, and fixed at the point at which a slight fall in tension indicated the onset of internal rupture. A third set was made to contract by plunging directly into barnacle Ringer's having KCl substituted for NaCl to make a total of 150 mM/liter K^+.

RESULTS

General Description of Fibrils

The average diameter of fibrils examined in the phase-contrast microscope after glycerinization

and maceration in barnacle Ringer's solution was 4μ, in the range 3.5 to 7 μ. The sarcomere lengths of different fibrils, or even parts of the same fibril, taken from the same fiber, varied considerably. Since the whole, fresh, living fiber shows a regular, banded appearance, it follows that during the glycerinization process partial local contraction occurs in some regions of the fibers, at the expense of others which are stretched out. These effects persist through to the isolated fibril stage. Examination of fibrils with different sarcomere lengths shows that the process of stretching is accompanied by an increase in the I band length whilst the process of shortening results in a reduction and eventual elimination of the I band (Fig. 1), as in rabbit psoas muscle (Huxley, 1957).

The evidence of local stretching and contraction, together with differences in observed A and I band lengths, makes it impossible to assign any definite values for sarcomere length and other parameters of normal fibrils at rest from glycerinated material. However, approximate average values can be assigned. The mean sarcomere length is about 8.5 μ and the A band about 5.5 μ, with values for the latter ranging from 4 to 8 μ.

H zones were not visible at all in most fibrils, no matter what the sarcomere length, and, when present, they were extremely indistinct. Even moderate to extreme stretching did not lead to the appearance of H zones.

Contracted Fibrils

Fresh, living fibers were made to contract in each of three different ways: maximal stimulation of the motor nerve; sudden immersion in Ringer's with 150 mM KCl (substituted for NaCl); sudden immersion in ice-cold 50 per cent glycerol. In the first case, 50 per cent glycerol at 0°C was quickly added to the fibers; the second set were withdrawn, contracted, and plunged quickly into 50 per cent glycerol at 0°C. Also, some fibrils were prepared in glycerol at body length and then induced to contract by adding ATP. In all cases, the 50 per cent glycerol was buffered with 0.67 M phosphate. After 24 hours in this solution at a temperature of −20°C, the fibers were transferred to fresh, buffered glycerol and kept at −20°C for 6 weeks.

Close examination revealed distinct differences between the fibrils contracted in each of these ways (Fig. 1). The differences were particularly marked between fibrils caused to contract by neural stimulation and fibrils caused to contract by ATP.

The most extensively contracted fibrils are those stimulated *via* the nerve. The zones of the most heavily contracted fibrils which were of greatest interest to us could be recognized by comparing them with the zones of the less heavily contracted ones, some of which are found in all the material. In these, the Z discs are still easily discernible. The sites of the Z bands were eventually always located with some degree of confidence. Characteristically, the fibrils still show a distinct, though faint, Z band, but this forms the center of a single, broad, uniformly dense contraction band. The middle regions of the sarcomeres, the centers of the old A bands, are now light in appearance. Almost as extensively contracted are the fibrils from KCl-treated fibers, but these show a variety of appearances ranging from one similar to that of the nerve-contracted fibrils, to one in which there are two distinct denser bands on either side of the Z disc. The latter is the characteristic appearance of fibrils made to contract by prior isolation followed by addition of ATP. In them there are two, sharply defined, dense contraction bands, clearly separated, on either side of the old Z band, which becomes extremely faint. Fibrils from fibers plunged directly into glycerol resemble those from KCl-treated samples; that is, there is a non-uniform single contraction band with the Z disc at its center, but three bands are distinctly visible in it: two are present at the outer margins and the other is the Z disc itself.

The reasons for these different appearances will become apparent after a detailed consideration of the electron microscope observations.

Nature of the Filaments and Connections

THICK FILAMENTS: The thick filaments are present in a hexagonal array (Fig. 5) as in many other muscles (Huxley and Hanson, 1960),but have a range of diameters between 150 and about 180 A. In some transverse sections, they show the hollow appearance often associated with thick filaments of arthropod muscle (Hodge, 1955; Smith, 1961; Bouligand, 1963). The thick filaments lie closer together than they do in rabbit psoas muscle, being separated by a distance only about equal to their own thickness.

THIN FILAMENTS: We have never observed regular arrays of thin filaments, which are not clearly defined in any of the material examined. Hence, it is not yet possible to assign a definite number to each thick filament. Their diameter is
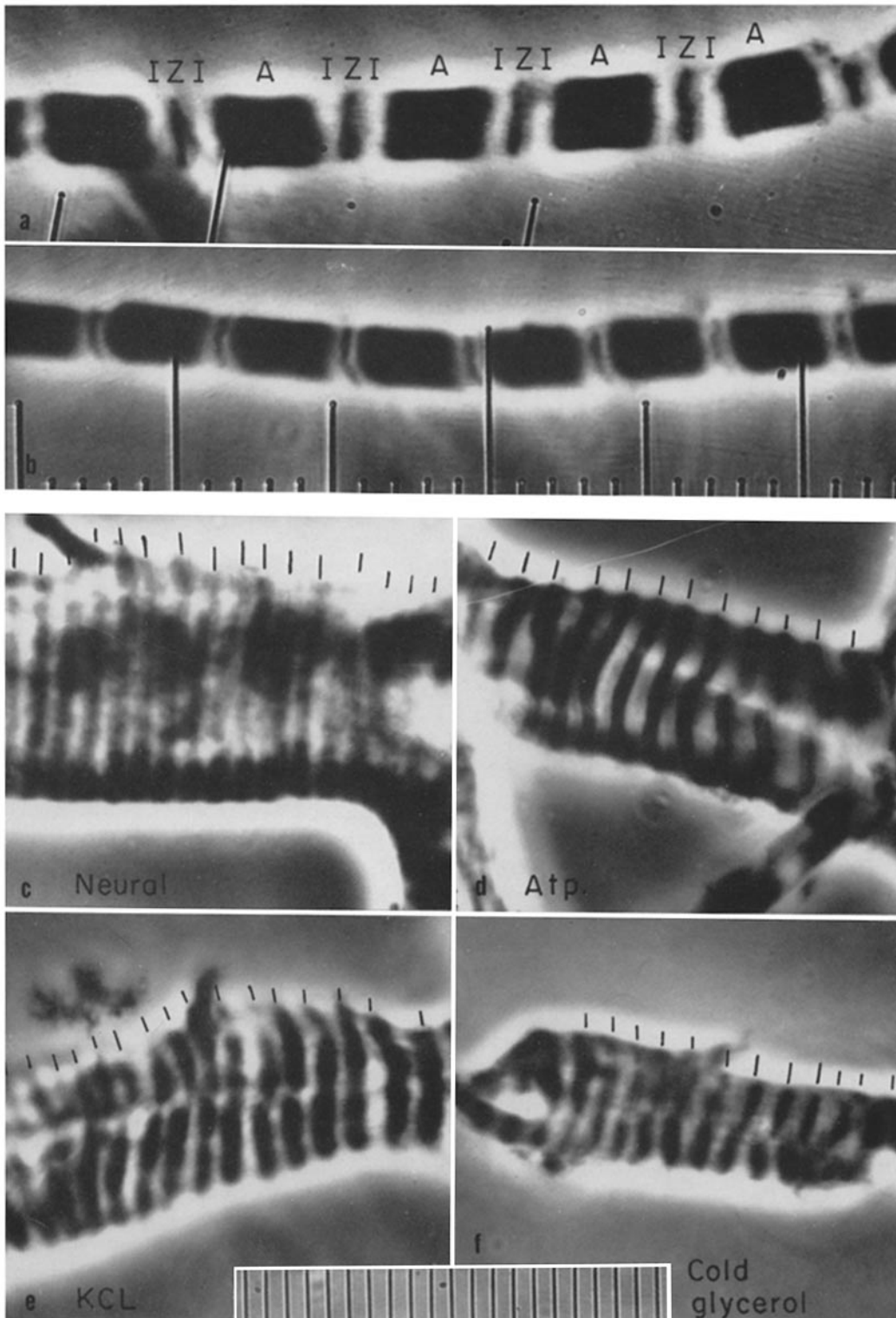
FIGURE 1   Phase-contrast light micrographs of relaxed or slightly stretched (a), partially contracted (b), and supercontracted (c to f) B. *nubilus* fibrils. Mode of stimulation to supercontraction: c, neural; d, ATP after glycerination; e, KCl; f, cold glycerol. Calibration: 2 μ per small division. Positions of Z discs indicated in c to f. Magnification: (a) and (b) = 2,450; (c to f) = 1,550.

about 60 A (Fig. 4). They are extremely long, as are the thick filaments, compared with those of rabbit psoas, being about 4 μ as seen in material stretched to 2$\times$ rest length (Fig. 3). Since the normal length of the sarcomeres is around 8 μ, the thin filaments must almost touch in the middle at rest-length, so that the absence of H zones is not surprising. However, H zones are not conspicuous even in stretched material, possibly because of the high density of the A bands resulting from the close spacing of the thick filaments.

BRIDGES: An array of short cross-bridges between thick and thin filaments is apparent (Fig. 2). In addition, the thin filaments give the appearance of being connected to each other by long cross-bridges in the regions within about 1 μ of the Z bands (Fig. 2). In the phase-contrast microscope these regions appear denser than the rest of the I band (Fig. 1 a).

Nature of the Z Discs

The Z discs of fibrils examined by light microscopy at rest length are about 0.3 μ thick when seen most sharply, i.e., at the limit of resolution. Longitudinal sections 250 to 650 A thick examined in the electron microscope show Z bands having a variety of appearances, but with two clearly distinct extremes. In one extreme, a continuous band stretches uninterrupted across an entire filament (Fig. 5 a). In the other extreme, the whole Z band is broken up into a number of dense bodies. The remainder are regarded as intermediate, and in them both strips and bodies may be seen (Fig. 5 b). Thin filaments run into the dense bodies. The apparent thickness of the continuous band is about 450 A. The thickness of the broken band may be almost twice this.

The dense bodies may be neatly aligned to form a band, but are often displaced by several hundred angstroms from the mean plane of the disc. Superficially, they appear ovoid in shape, with the long axis always parallel to the long axis of the fiber. They vary in size, from a minimum of 120 by 200 A to about 400 by 900 A.

In ultrathin transverse sections which have included parts of a Z disc, the dense bodies appear even more varied in shape. This is apparently caused by the section's passing through bridges which connect the dense bodies together. The bridges must be highly elastic. Dense bodies which may be comparable to those seen in B. nubilus were first observed in invertebrate muscles by Kawaguti

and Ikemoto (1957) in molluscan muscle, who termed them J bodies. Hanson and Lowy (1961) in a paper on an oyster muscle referred to them simply as dense bodies.

Evidence will be presented later in this paper to show that the B. nubilus Z discs undergo an active, reversible change during excitation. Our present view is that the thin continuous band, which shows a slight zigzag arrangement of subunits and is not markedly different from the electron microscopic appearance of the Z discs of ordinary vertebrate skeletal muscles, represents the normal, unexcited state of the Z disc. However, we cannot rule out the possibility that this state represents the normal for only some of the fibrils. Some excitation of the muscle fibers is inevitable with all of the methods used. We are attempting to resolve the problem by first treating the muscles with a calcium-chelating agent, EDTA, which renders the fibers completely unexcitable. If our view is substantiated, the disc composed entirely of separated dense bodies must be taken to represent the fully activated state. The intermediates could be showing stages of transformation in which only parts have changed from unexcited to excited states. In the transformation, elemental units would either rearrange, or perhaps merely twist through 90°.

Sequence of Events During Contraction

The fate of the long thin filaments at the center of the fiber during contraction and supercontraction is at present not known to us. There is sometimes a dark band in the center of the sarcomere in partly contracted fibrils seen in the phase microscope, and such a band may appear during ATP-induced contractions of glycerinated fibrils. It could be due to a pile-up or overlap of thin filaments.

The fate of the thick filaments depends in a profound way on the manner of stimulation of the muscle. We will deal first with events occurring in the normal muscle in the body. The thick filaments do not shorten, during contraction under tension, to below 50 per cent rest length (McAlear et al., 1965). Instead, they move towards the Z bands with a consequent shrinking and eventual obliteration of the I bands (Fig. 1).

NEURALLY EVOKED CONTRACTIONS: When the filaments reach the Z bands, the majority evidently pass through into the adjacent sarcomere, in contractions induced by neural

FIGURE 2 Electron micrograph of longitudinal ultrathin section of partially contracted fibril in region of Z disc. Note: thick filaments of A band with cross-bridges attached; cross-bridges between thin filaments in I band; Z disc partially broken up into continuous bands and separate dense bodies. Living fiber dissected free and held at rest-length during sudden immersion in OsO_4 fixative. $\times$ 90,000.
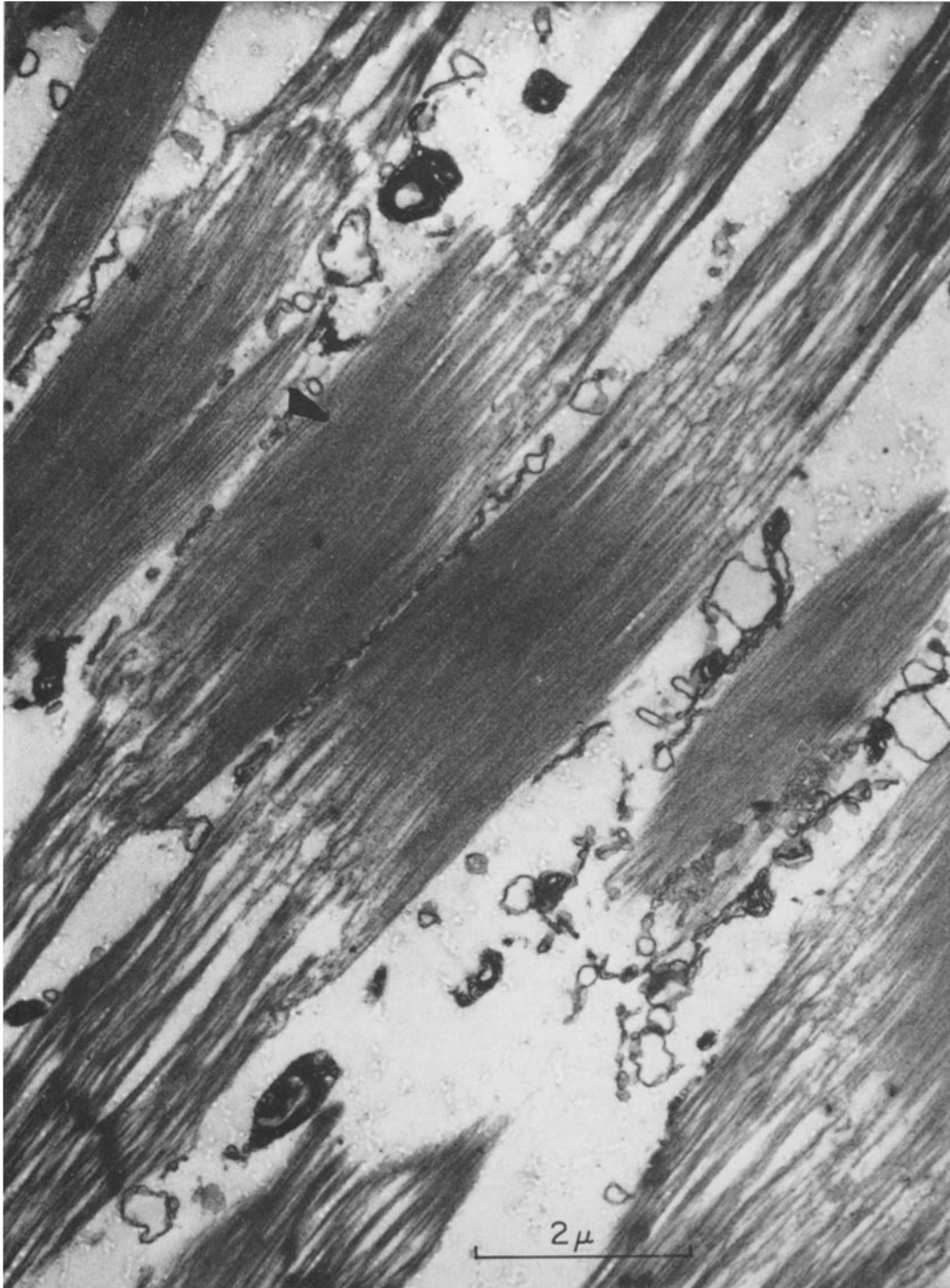
FIGURE 3   Longitudinal section of *B. nubilus* fibrils from muscle stretched just to breaking point. Living fiber stretched in air whilst monitoring tension continually, covered quickly by OsO₄ fixative. × 16,000.

stimulation (Fig. 6). A few are seen bent back at the region of the dense bodies. No evidence of any form of coiling or thickening of the thick filaments at the Z band has been found.

The spatial arrangement of the thick filaments passing through the Z disc is of particular interest. If the thin filaments are pulling on individual thick filaments right up to the disc, it is difficult to see how they can retain a regular arrangement there. Some thin filaments can be seen among the thick ones, and in many sections of supercontracted fibers the thick filaments which have passed through present an orderly appearance. The passed-through filaments are a variable distance apart, but in clusters of thick filaments examined close to dense bodies of the Z disc the separations are similar to those obtaining in the A band at rest-length, and the same hexagonal array is evident. The regular hexagonal array is, however, broken down in the case of the majority of thick filaments passing through. The occasional regularity suggests that some filaments pass through as small groups retaining their original spatial arrangements, rather than singly. Fig. 7 *b* is a transverse section of a supercontracted fiber through part of a Z disc. A section through the center of the A band of the same material is also shown (Fig. 7 *c*). Here, the thick filaments are still seen in regular array.

The thick filaments overlap as they pass through the Z disc, thereby forming a dense contraction band about the disc (Fig. 8). The band thickens as the contraction proceeds and the filaments pass through farther. At the same time, the lateral expansion of the fiber causes a moving apart of filaments, or rather groups of filaments, in the A band, which lightens.

The result of contraction, then, is first of all a shortening of the I bands to obliteration, followed immediately by the appearance of contraction bands, as thick filaments overlap across the Z disc.

NON-NEURALLY EVOKED CONTRACTIONS:

Contractions occurring as a result of agents other than the motor nerve acting on intact fibers, or obtained by adding ATP to glycerinated material, although differing in details, have in common the fact that in them extensive passing through the Z discs by thick filaments rarely occurs.

In the preliminary report (Hoyle and McAlear, 1963), we published a picture of a glycerinated fibril contracted by ATP in which we claimed that overlap of thick filaments across the Z disc had occurred. This particular fibril was selected because it was observed to contract unaccompanied by complex banding changes. It could, indeed, have been the subject of extensive passing through, as we suggested. But since we know now that this is an extremely rare occurrence in glycerinated material, and since the fibril in question was not examined electron microscopically, the appearance could have been due to heavy crumpling of thick filaments at the Z disc, but not passing through. It is now abundantly evident that studies of glycerinated fibrils in the light microscope can be highly misleading and should be accompanied by electron microscope studies of the same material.

In KCl-treated fibers a partial penetration occurs, but in material previously glycerinated and then treated with ATP very little or even no passing through may be discerned in most of the fibrils. The contractions are accompanied instead by a bending back of the thick filaments at a region close to the Z disc (Fig. 9). The regions of bending back form dense bands which first appear about 1 μ distant from the Z disc on either side, but eventually merge with the disc. It is apparent that, under these conditions, the movements of the filaments are not coordinated with changes in the Z discs, which are, in any case, imperfect.

Also, in these unnatural contractions the A band appears to shorten (Baskin and Wiese, 1964). This phenomenon is, however, an artifact and will be fully explained in a subsequent paper (McAlear *et al.*, 1965).

FIGURE 4   Transverse section through muscle stretched 150 per cent. The section goes through part of a Z disc (lower left), I band, and overlap zone (main body of micrograph). Few thin filaments are seen clearly in the overlap zone, but, where distinct, they have a diameter of 60 A. Thick filaments have a diameter of 180 A. Living specimen stretched in barnacle Ringer's which was quickly replaced by ice-cold glutaraldehyde. Fixed in OsO$_4$ and stained with uranyl nitrate and lead citrate. Embedded in Epon. X 60,000.
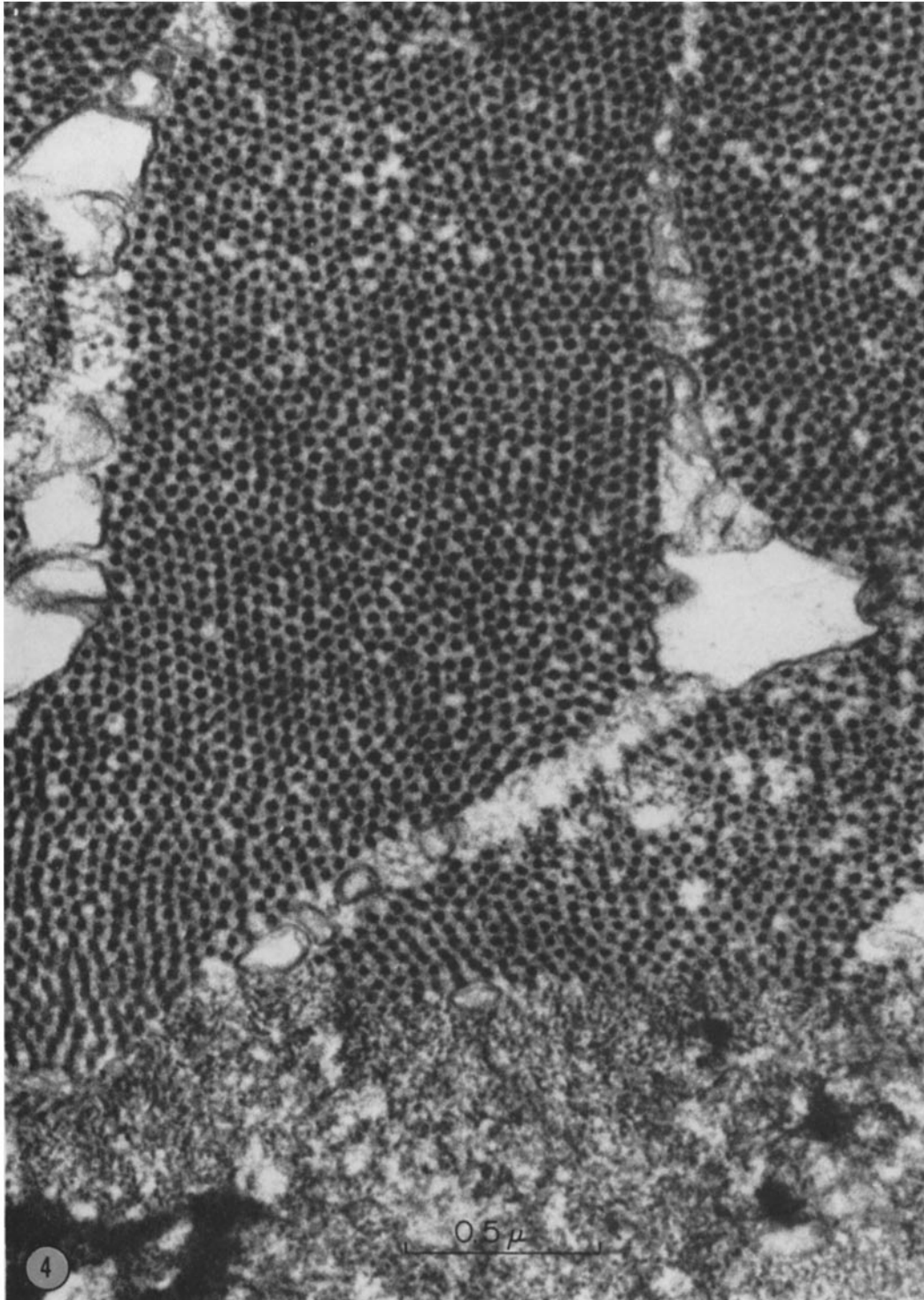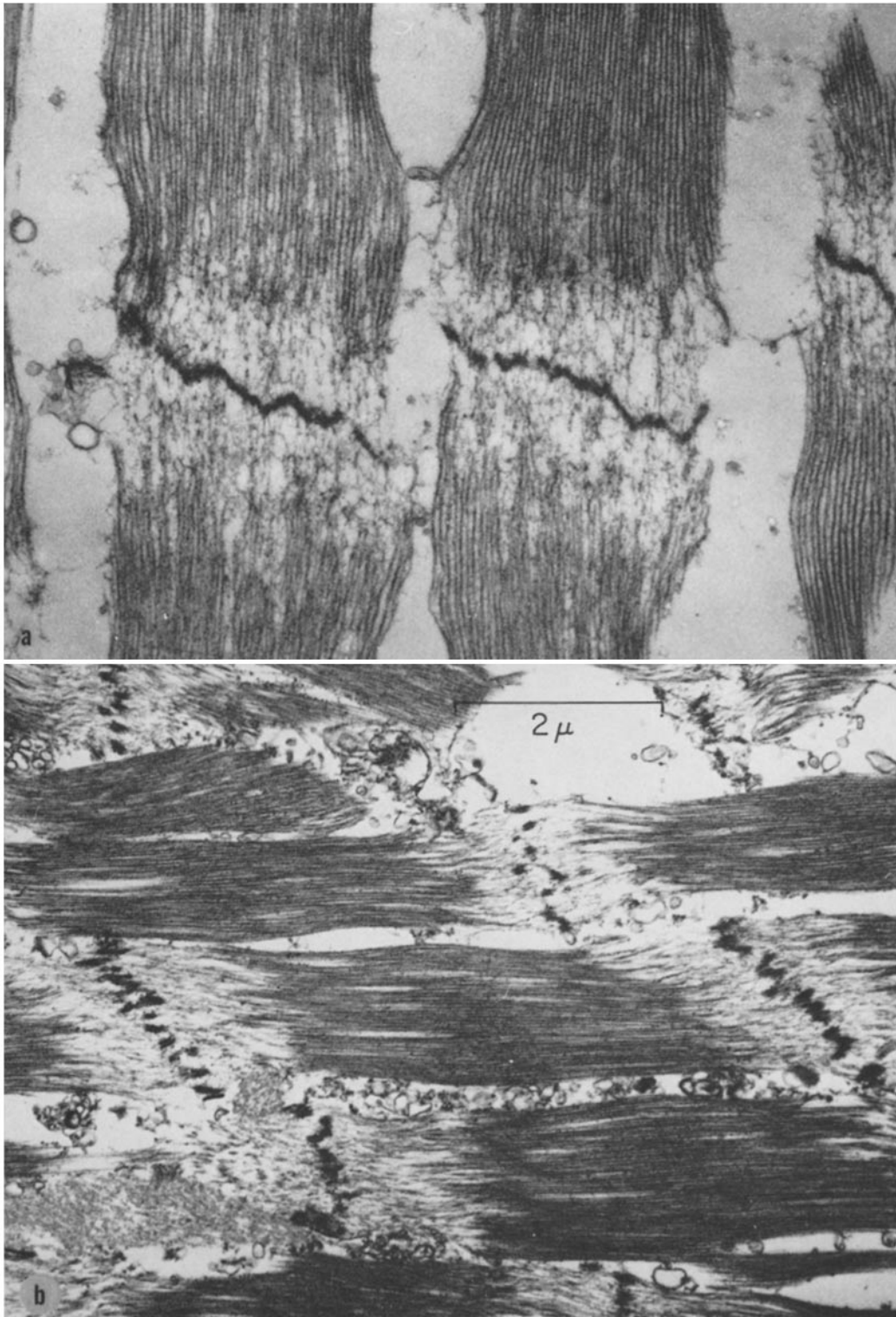
FIGURE 5   Two appearances of Z discs. *a*, Considered to represent the resting condition in which the Z disc is continuous across the fibril. *b*, Considered to represent fully activated condition in which disc is completely broken up into dense bodies with spaces between them. From same material fresh-fixed in OsO_4. × 16,000.
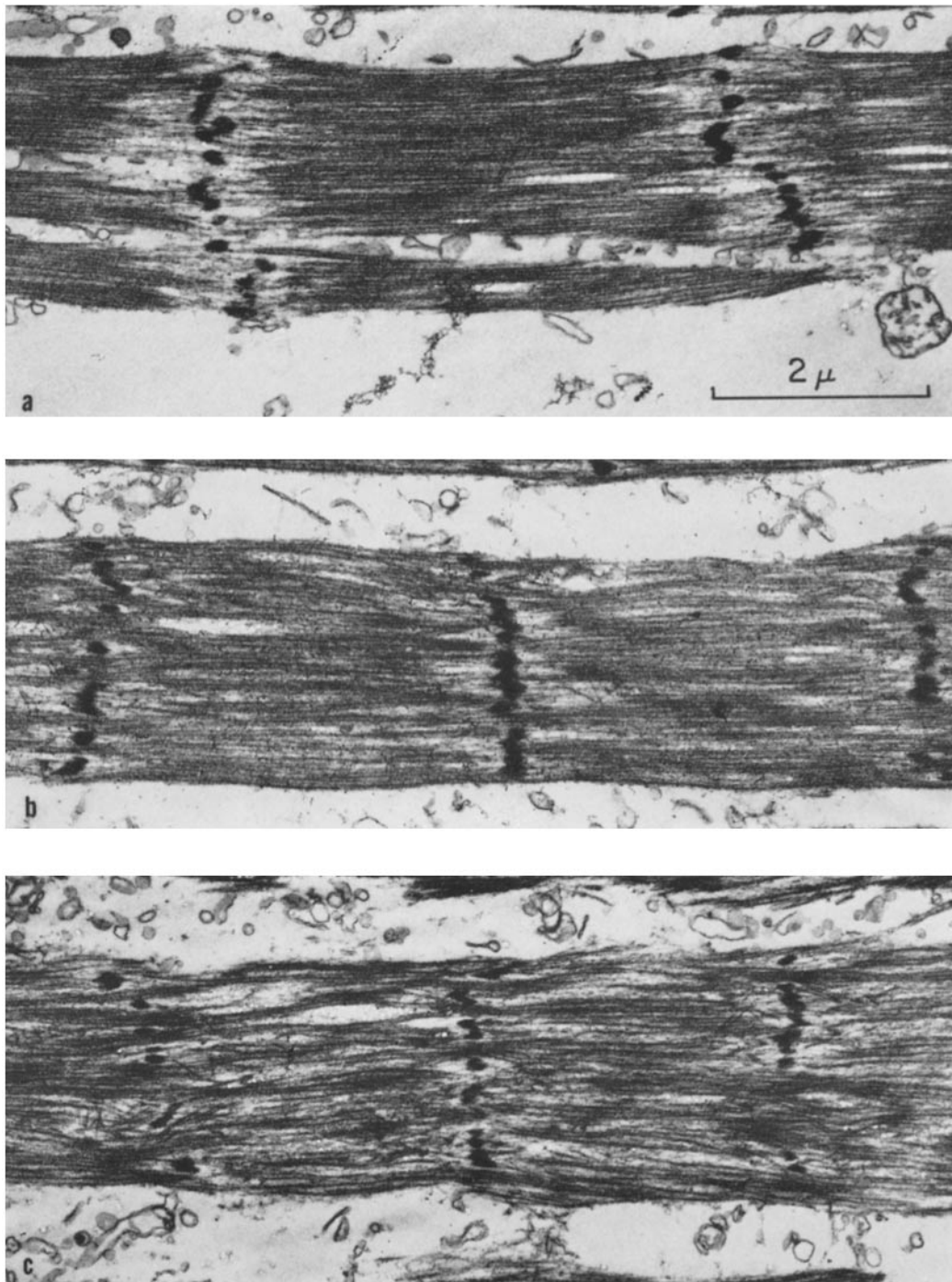
FIGURE 6   Stages in development of supercontraction. *a*, Contraction to 60 per cent rest length. Thick filaments have invaded A band, almost completely, *b*, Contraction to 50 per cent rest length. Thick filaments have just penetrated the spaces developed in the Z discs. *c*, Contraction to 40 per cent rest length. Further interdigitation of thick filaments from adjacent sarcomeres has occurred across the perforated Z disc. Similar muscles all excited to contract by neural stimulation whilst being held at fixed lengths and covered quickly with OsO₄ fixative at peak force. × 16,000.

Process of Z Disc Expansion

It is to be expected that the Z discs will become expanded passively as the fibril thickens during contraction provided: there is a barrier to the movement of water out from within the fibril; the contractile filaments move apart during contraction; the thick filaments are actively forced across the Z discs. It is a fact that the whole sarcomere thickens during contraction and the Z discs certainly expand. Several findings prompt us to suggest, however, that the Z disc expansion is not simply a passive process.

The Z discs expand even in glycerinated material in which the basic structure of the fiber has been considerably disrupted. On the other hand, in such material the thick filaments seldom pass through. Such fibrils are not under tension, and this may affect the horizontal alignment of the thick filaments. Alternatively, it may be that in such fibrils either the Z disc does not expand sufficiently, or the timing of the expansion is not coordinated correctly with respect to the movement of filaments. The latter possibility implies an active Z disc expansion resulting from adding ATP to the glycerinated material. This possibility is open to experimental test, by varying the ATP and other ionic concentrations of the activating medium, for if Z disc expansion and contraction are independent processes, they might be differently affected. Experiments with different ATP concentrations were carried out and gave surprising and dramatic results.

It was found that high concentrations of ATP (0.6 M—other ions normal) routinely cause the Z discs to expand faster than the fibril contracts. This happens also in lower concentrations of ATP in about 3 per cent of the fibrils, so it is not necessarily an artifact caused by a chelating action at high concentrations. Since ATP-induced contractions of glycerinated fibrils are slow compared with those of fresh muscle the process can be filmed. Some shots from a movie film showing this expansion process are given in Fig. 10 *b* to *i*. Note

that the fibrils acquire a beaded appearance as the Z discs expand. Shortening develops to only about 45 per cent rest length, by which time the Z discs have almost vanished, perhaps dissolving in the solution.

We have made electron micrographs of such fibrils (Fig. 10 *a*), and they give a clear impression of the occurrence of an explosive expansion of the Z disc.

The question of how this expansion is brought about is an intriguing one awaiting future research. The parallel question as to how selective excitation of the Z disc may be brought about in the intact muscle must also be raised. Recently, a system of tubules, termed Z tubules, connecting the exterior specifically with the Z discs has been discovered in crab muscle by Peachey (1964, and in press), and it is tempting to suppose that a similar system exists in *B. nubilus* with the function of stimulating the Z disc expansion process.

Uniformity of Contractions

The muscle fibers of the retractor muscle receive a dual axon supply, each giving off multiterminal innervation. The neuromuscular electrical events are primarily local postsynaptic potentials, with secondary graded responses (Hoyle and Smyth, 1963) superimposed. The force of the contractions is a function of the frequency of excitation of the nerve. For this kind of neuromuscular mechanism, which is common in arthropods, it is not known what detailed muscular events are associated with graded contractions. Three possibilities exist: 1. a variable number of fibrils give all-or-nothing contractions; 2. fibrils contract unevenly: some regions go to a full extent of contraction, whilst neighboring ones get extended (even under isometric conditions) or remain only partially shortened; 3. the contractions of the sarcomeres are themselves graded and occur to approximately the same extent along the whole length of the fibrils.

We observed that different fibrils prepared

FIGURE 7   Nature of passing-through of thick filaments in contracted muscle. Longitudinal and transverse sections across the Z disc region (*a* and *b*) and center of A band (*c*), all from same block, though *c* probably from a sarcomere less contracted than *a* and *b*. Note in *b*: section has included part of Z disc. Thick filaments in clusters retaining hexagonal array pass through gaps between dense bodies of disc. Muscle stimulated to contract *via* nerve, then covered quickly with OsO₄ fixative. X 90,000.
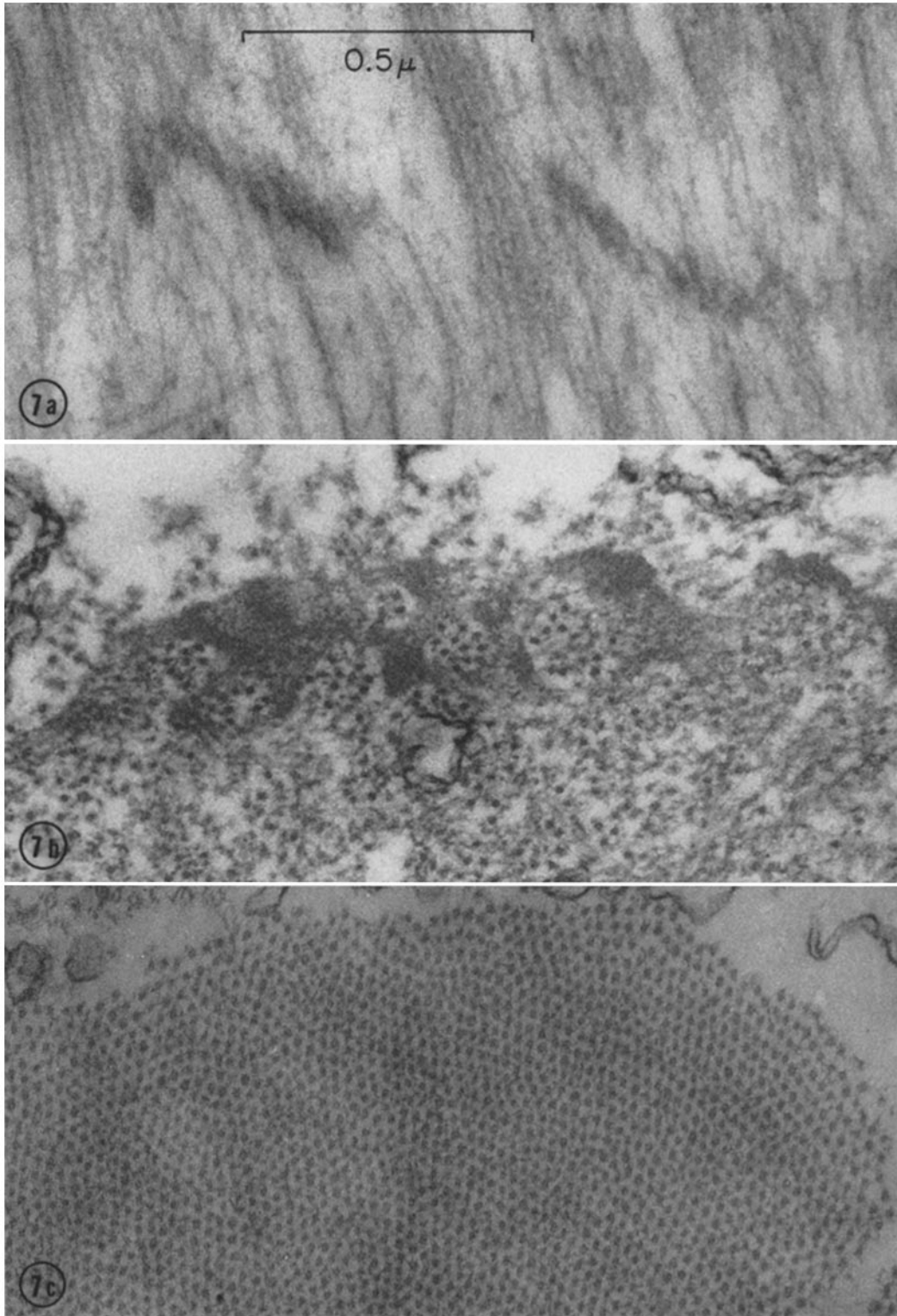
FIGURE 8 Nature of normal, neurally evoked supercontraction. Part of the Z disc of a supercontracted fibril showing extensive interdigitation of thick filaments from adjacent sarcomeres. Muscle was stimulated *via* the nerve and allowed to supercontract under light load. It was immersed quickly in OsO₄ fixative at maximum contraction. × 90,000.
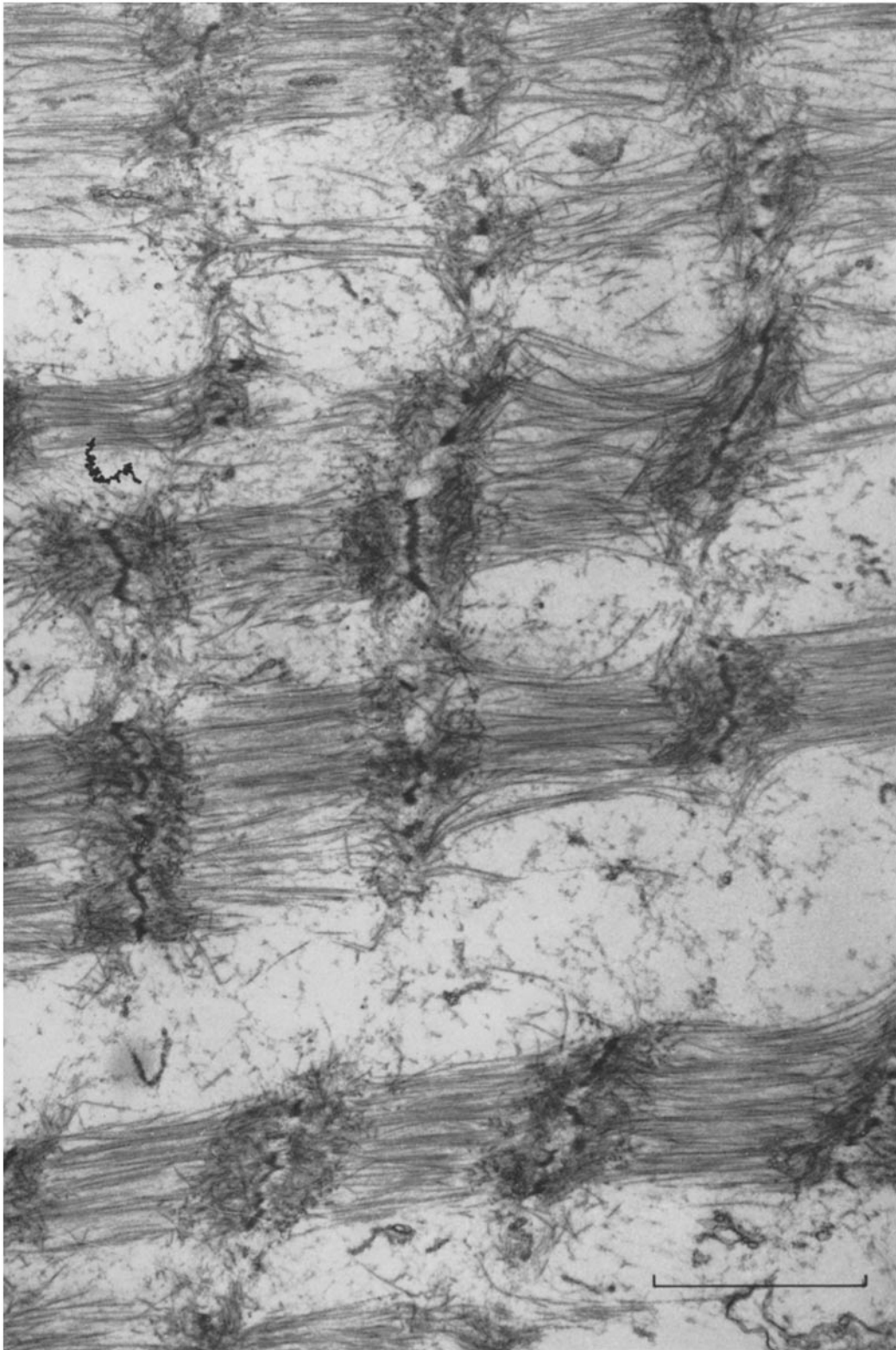
FIGURE 9 Anomalous supercontractions of glycerinated fibrils caused by adding ATP. Note that most of the thick filaments have bent back in the Z regions instead of going through as in normal supercontraction. × 16,000. Bar = 2 μ.
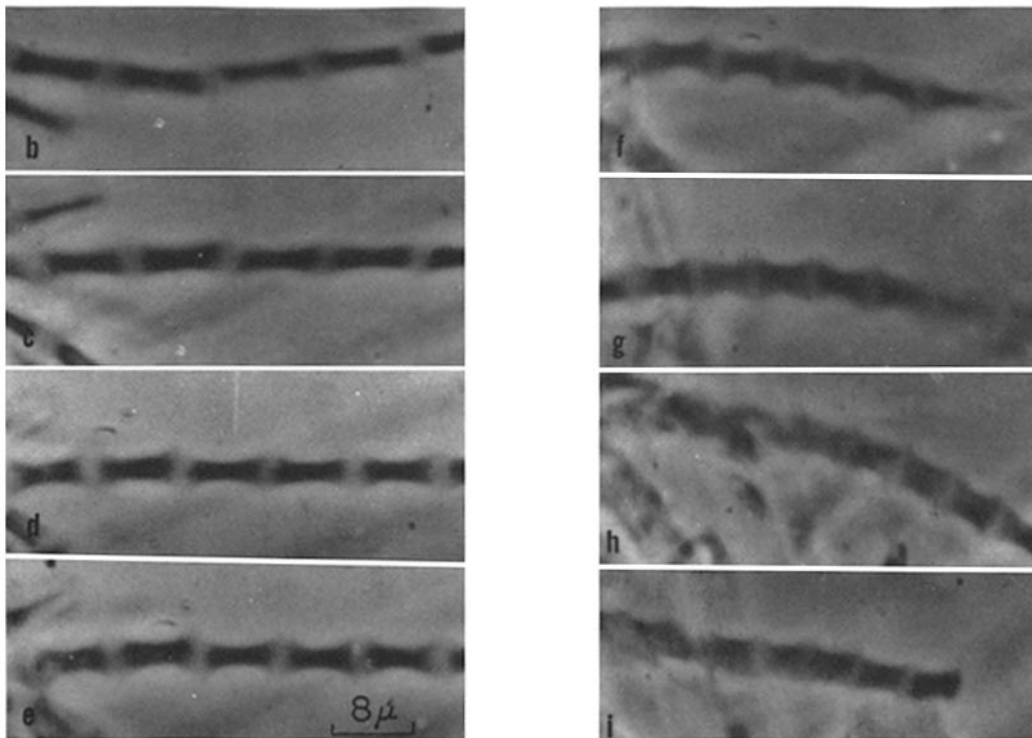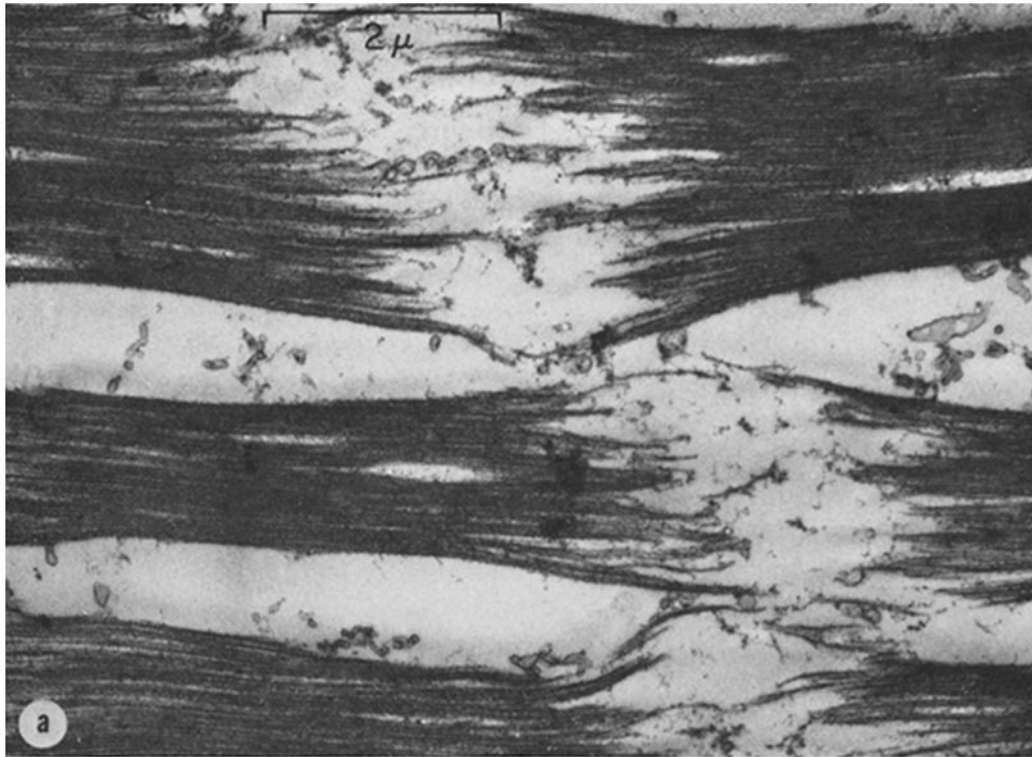
FIGURE 10   Expansion of Z disc. *a*, Exploded Z discs of glycerinated fibrils treated with 0.6 M ATP. × 16,000. *b* to *i*, frames from movie showing Z disc expansion and shortening of glycerinated fibril after adding 0.6 M ATP.

after glycerinization following neurally evoked contractions always showed a wide range of sarcomere contractions. Furthermore, contractions were different in different parts of the same fibril. Unfortunately, we cannot rule out the possibility that secondary local or general relaxations may have occurred. However, the observations strongly suggest that local variations in extent of shortening along the length of individual fibrils play a major role in achieving graded contractions, thereby supporting the second of the three alternatives presented above.

DISCUSSION

The results of the present work provide a suitable basis for explaining supercontraction down to levels of about ⅛ rest length without requiring marked changes in the effective length of thick filaments such as have been postulated to occur when the sarcomere length is reduced below the A band length. The results are not only compatible with the sliding filament hypothesis, but they provide critical confirmatory support for its general validity. There is no evidence, however, that the thick filaments of rabbit psoas muscle can pass through the Z bands. Two possible explanations of the lack of capacity for supercontraction in many vertebrate muscles come to mind. The nature and functional capabilities of the Z discs are apparently different in the two cases. The Z discs of vertebrate muscle cannot, apparently, transform to the stage which is perforated and shows the dense bodies, in those examples studied so far (*e.g.*, Franzini-Armstrong and Porter, 1964). However, the appearance of the broken Z discs is not confined to *B. nubilus* but has been observed in several muscles of invertebrates, for example, the heart muscle of *Helix* (North, 1963) and skeletal muscle of *Limulus* (de Villafranca, 1961), though the appearance has not previously been linked with the possibility of supercontraction.

One invertebrate muscle containing dense bodies to which thin filaments are attached, the gray oyster adductor, is supercontractile but is not striated muscle in the ordinary sense. Here the dense bodies are present in a consecutive series of V's, giving the appearance of a double-oblique situation in the whole fiber. *B. nubilus* fibers, by contrast, satisfy all the classical requirements for definition as striated.

Thus, the barnacle muscle may, in its structure, bridge the gap between ordinary striated muscle and non-striated muscle, by having highly mobile components in the Z disc, yet retaining them in some semblance of a plane.

It is necessary only to displace the *B. nubilus* dense bodies and disperse them randomly throughout the fibril to convert a "striated" fibril into an unstriated one. Dense bodies have been observed in mammalian smooth muscles (Mark, 1956). The most significant special feature of smooth muscle is its immense contractibility.

Of course, the converse type of displacement could be considered the more probable event in evolution. That is, primitive muscle may be regarded as formed of scattered Z bodies to which thin filaments are attached, oppositely polarized in the two directions in the long axis. Intermingled with them are other filaments oppositely polarized in two halves, which make functional crossbridges with the primary set. Evolution of fast-contracting striated muscle may have involved a combination of lateral alignment of the dense bodies, which would, as it were, become Z bodies, with the development of connecting elements.

The existence of supercontraction may be called into question as a phenomenon occurring during normal life. There is no doubt, however, that any strong prod applied to the scutal plates is followed reflexly by contractions down to about ⅓ rest-length (Fig. 11). The function appears to be to eject a defensive squirt of water from within the body cavity out through the plates. We have studied these contractions by cutting a "window" in the shell overlying a scutal depressor muscle in an otherwise intact animal. They undoubtedly involve passing through the Z disc of thick filaments and they can occur repetitively, apparently indefinitely. As we have noted above, there is a tendency for thick filaments to remain together in hexagonally arrayed clusters during these processes, but many must tear apart and some become bent back. The stress on the thick filaments in *B. nubilus* muscle from the moment of initiation of force development must be such as to disrupt the hexagonal array; this follows from the irregular nature of the Z discs. The tendency to disruption will increase as contraction proceeds. It must be concluded that the thick filaments are held together laterally by bonds which are elastic. One may inquire whether there is any electron microscopic evidence for such bonds?

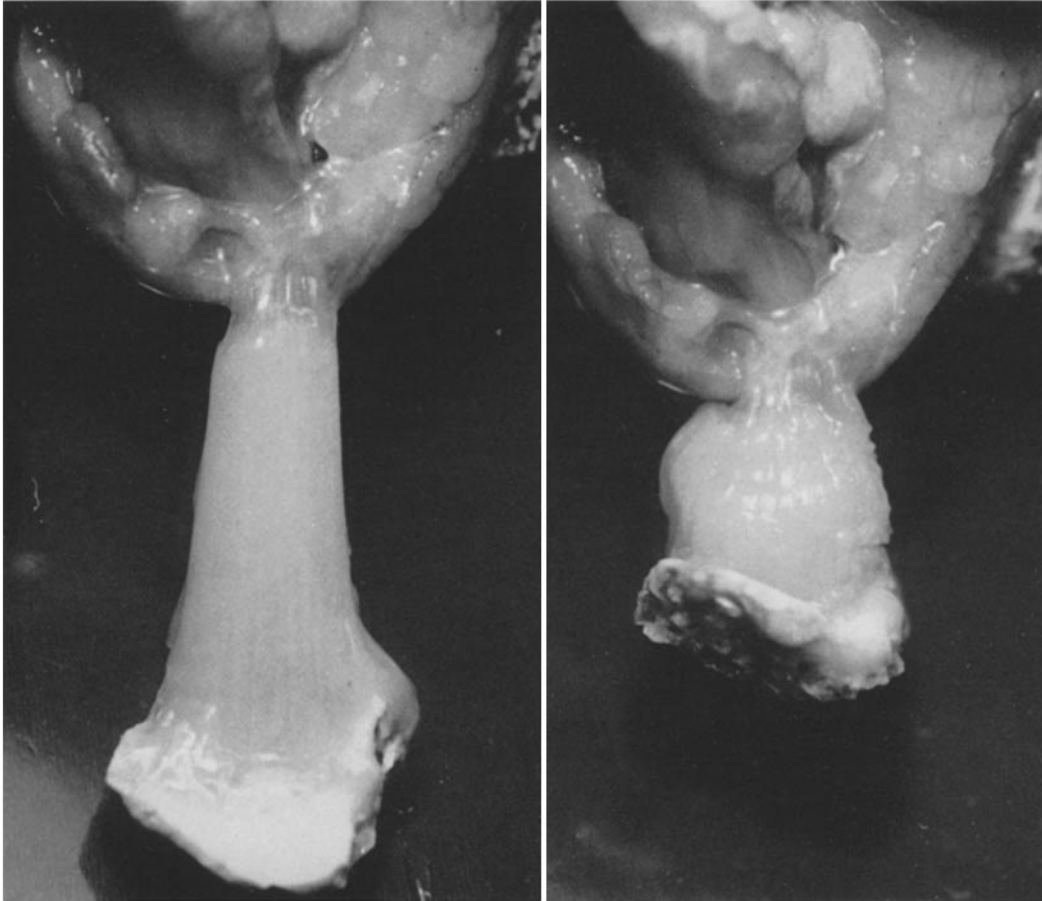Undoubtedly lateral projections from the thick filaments occur at approximately regular in-

FIGURE 11   Natural contraction of scutal depressor muscle of *B. nubilus*. The basis was cut through in the region of attachment of this muscle and the attachment region freed. The shell surrounding the opening was then snipped away until the muscle could be bent outwards and its tendon end viewed from the inside. The rest of the animal was intact. The basal attachment was rested on the bench (left) with the muscle at a length at which the fibers were just taut (rest length). Contractions occurred from time to time spontaneously, and could be evoked reflexly by touching the tissue overlying the margin of the scutal plate. One such contraction is shown on the right. Still greater contractions have been observed. The length of the muscle was 4 cm; fibers are almost 2 mm thick. The contractions were photographed in air. Magnification, 1¼.

tervals, but it is not clear, in our *B. nubilus* preparations, whether these are all similar to the familiar bridges discovered by Huxley (1957) to occur between actin and myosin, or are some other kind. We have no evidence to rule out the possiblity of some of them being bridges between thick filaments.

Indeed, two sorts of bridges may occur: one between actin and myosin, the other between myosin and myosin. The latter are probably disrupted during the process of glycerinization

(McAlear *et al.*, 1965). During contraction, the latter type of bridge would become extended as the fiber thickens, breaking at the outer ends of many of the fibrils as these move apart and pass through the Z discs. Presumably, they would be reformed during the recovery process. The existence of myosin-myosin bridges would settle the still unresolved general problem of how alignment of the fibrils within the A band is maintained in striated muscles.

A final, pertinent question is whether or not

supercontraction occurs in other striated muscles. A number of other invertebrate muscles in diverse phyla have striated muscles which can shorten extensively, for example, the subumbrella muscles of medusae (Krasinska, 1914) and the "gizzard" of *Syllis* (Haswell, 1889). Supercontractions have been observed in several living insect muscles (personal, unpublished observations), and an overlap of thick filaments has recently been claimed as a basis for supercontraction in isolated fibrils obtained from *Drosophila* flight muscle

(Aronson, 1963). It thus seems that the phenomena described in this paper may be quite widespread.

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