THE INFLUENCE OF FIXATION UPON THE FINE STRUCTURE OF THE Z-DISK OF RAT STRIATED MUSCLE

D. N. LANDON

M.R.C. Research Group in Applied Neurobiology, Institute of Neurology, Queen Square, London, W.C. 1, England

SUMMARY

The fine structure of the Z-disk in rat striated muscle has been found to be dependent upon the manner of its fixation. Material primarily fixed in osmium tetroxide shows a square or 'woven' lattice in transverse sections, with a spacing of around 22 nm, and with its axes off-set by 45° from the axes of alignment of the files of I-filaments prior to their entry into the Z-disk. In longitudinal sections the disk may have a zig-zag appearance with individual I-filaments of one sarcomere apparently linked obliquely to 2 or more I-filaments of the next. Transverse sections of Z-disks in muscle primarily fixed in glutaraldehyde show a square-lattice pattern with a spacing of about 11 nm, its axes coinciding with the axes of alignment of the adjacent I-filaments. In longitudinal sections oblique linkages of the ends of the I-filaments are not seen, and they appear either to interdigitate, or to be longitudinally continuous with those of the next sarcomere. This second fine structure is interpreted as being the consequence of the superimposition of 2 basic square lattices, each of 22-nm period, 50% out of register along each axis, and each formed from the lateral linkage of the ends of the I-filaments with the ends of adjacent filaments from their own sarcomere. The relationship of this postulated structure to the lattice patterns previously described, the nature of the cross-linking material, and some possible functional implications are briefly discussed.

INTRODUCTION

The many investigations into the fine structure of the sarcomere of striated muscle, and into the biochemistry of its component parts, have yielded extensive support for the concepts embodied in the sliding filament model system for muscular contraction proposed by Huxley & Hanson (1954) and Huxley & Niedergerke (1954). A feature which has attracted less attention than have the fibrillar components of the sarcomere is the Z-disk (or line), despite the fact that its structure is of the first importance to the functioning of the sliding filament model, since the Z-disks are considered to form the fixed points at the ends of the sarcomere to which the actin filaments are attached, and upon which they act during contraction.

The Z-disk has been examined electron-microscopically by several workers including Knappes & Carlsen (1962), Huxley (1963), Franzini-Armstrong & Porter (1964), Reedy (1964) and Kelly (1967). From these studies there have emerged models of Z-disk structure which, whilst they differ in certain details of the interpretation of features seen in longitudinal sections, in general agree that the disk seen en face in transverse sections presents the appearance of a square lattice with a period...
of approximately 22 nm and composed of 5-nm filaments. Huxley (1963) has pointed out that this lattice shows a striking similarity to the crystal lattice of tropomyosin B and, despite failure of attempts to identify tropomyosin B in Z-disks by other means (Pepe, 1966), this similarity of structure has been generally assumed to be evidence that tropomyosin B is a significant structural component of Z-disks.

It will be demonstrated here that the fine structure of the Z-disk can be observed to vary both with the method of fixation employed and, to a lesser extent, with muscle fibre type. These observations will be discussed in relation to previous fine-structural models, to the validity of the identification of tropomyosin B in Z-disks on structural grounds alone, and to another possible functional role for the Z-disk in muscular contraction.

MATERIALS AND METHODS

The tissue used in this study was obtained from the ventral half of the 4th-8th internal intercostal muscles of albino rats weighing approximately 250 g. Muscle from this particular site was chosen for its ease of access and fixation, its conveniently mixed population of small mitochondria-rich and large mitochondria-poor fibres, and for the lack of any necessity for elaborate clamps to maintain fibre length and orientation during fixation. The animals were anaesthetized with ether and the major part of one side of the chest wall was then rapidly excised with scissors and pinned out under slight tension, inner side uppermost. Fixation was effected by rapid infiltration of cold (4 °C) fixative through a fine needle into all layers of the intercostal spaces in sufficient quantity to produce slight distension of each space. After 10 min small portions of the internal intercostal muscle were excised and transferred to fresh fixative.

The following variations of fixation procedure were employed: (i) infiltration and further fixation for 30 min with 4 % glutaraldehyde solution adjusted to pH 7.4 with a 0.1 M phosphate buffer, followed by post fixation for 2 h in Millonig’s (1962) 1 %, phosphate-buffered, osmium tetroxide solution, (ii) Infiltration and subsequent further fixation for 2 h with 4 % glutaraldehyde adjusted to pH 7.4 with a 0.1 M phosphate buffer. (iii) Infiltration and subsequent further fixation for 2 h with Millonig’s (1962) 1 %, phosphate-buffered, osmium tetroxide solution. (iv) As (i), but after the lapse of a measured time interval between excision of the chest wall and infiltration with the fixative. Specimens were obtained with post-mortem prefixed delays of 3, 10 and 20 min.

In the subsequent processing of the tissue, half of the specimens obtained by each fixation method were dehydrated in ethanol, the remainder in acetone. The dehydration was rapid, 45 min, and was followed by 15 min in 1,2-epoxypropane, and embedment in Araldite. The tissue blocks were cut with glass knives on a Cambridge (A. F. Huxley) ultramicrotome, and the sections collected on 400-mesh grids without support films; these were stained with uranyl acetate (25 min in a saturated solution in 50 % ethanol), and lead citrate (2 % solution in 0.1 N carbonate-free sodium hydroxide for 15 min). The stained sections were given a light coating of carbon and examined in an RCA EMU 3 G electron microscope at 100 kV. A tilting stage was employed to obtain stereo-micrographs using tilt angles of between 10 and 35°. The instrument magnification settings employed were calibrated with a 2160 lines/mm grating replica; these values were reproduced thereafter with an error of less than 5 %, by ‘normalizing’ the intermediate/projector lens systems prior to photography.

RESULTS

Tissue fixed in glutaraldehyde and subsequently post-fixed in osmium tetroxide, method (i): dehydration in ethanol

The structural details of the Z-disk in longitudinal sections of muscle fibrils have been found to vary with change in the relationship between the plane of section and the
planes of lateral alignment of the I-filaments as they enter the disk. Two clearly defined and different configurations have been observed in this material and the conversion of the one into the other in the same Z-disk, by the device of tilting the microscope stage about the long axis of the fibril, has demonstrated that these images represent alternative views of the same fine structure. The I-filaments of the adjacent sarcomeres appeared either to be in longitudinal continuity across the disk, or to overlap and interdigitate with those from the next sarcomere (Figs. 1, 2). Intermediate orientations of the disk resulted in confused images in which the I-filaments lost their identity upon entering the disk, their lateral edges merging to form an amorphous band.

The extent of overlap of the ends of the I-filaments within the disk was found to be consistently greater in the small diameter, mitochondria-rich muscle fibres (being 60–80 nm), when compared with that seen in immediately adjacent large-diameter, mitochondria-poor muscle fibres in the same section (20–30 nm) (Figs. 3, 4). In both varieties of fibre there was a considerable increase in the electron density of the I-filaments throughout their zone of overlap within the disk, and this additional density extended back along the filaments for some 30 nm into their own sarcomere.

In transverse sections of muscle fibrils, where the plane of section exactly coincided with that of the Z-disk, this was seen to be composed of a regular square lattice of filaments 5 nm thick, spaced at intervals of 11–11.5 nm. This square-lattice structure is seldom wholly regular over the whole cross-sectional area of the Z-disk, usually being divided into smaller areas, their principal axes at varying angles one to another and the discontinuities between the adjacent portions appearing analogous to grain boundaries in a crystal lattice (Fig. 5). Examination of sections inclined at a small angle to the plane of the disk, viewed either singly or as stereo-pairs (obtained by tilting the specimen between successive micrographs), has demonstrated that the small, regular 11-nm lattice is the product of the superimposition of 2 larger lattices of twice that period (i.e. 22 nm), the one out of register with the other by half its repeat distances along each axis. Where such slightly oblique sections passed from the Z-disk into the adjacent I-band it was observed that the I-filaments lost the haphazard arrangement which pertains throughout the major part of the I-band and, for a short distance prior to their entry into the disk, became aligned into a regular square array of filaments spaced 22–24 nm apart, this region corresponding to the zone of increased density of the terminal portions of the I-filaments which has been described above (Fig. 6). Each of the 2 basic lattices composing the Z-disk appeared to be formed from the cross linkage of the denser terminal portions of the I-filaments throughout the thickness of the disk, each with its immediate neighbours from the same sarcomere, the interdigitation of these I-filaments with those from the adjacent sarcomere determining the registration of the 2 lattices one with another. This structure results in the axes of the 2 lattices, and therefore of the combined fine lattice, coinciding with the axes of lateral alignment of the I-filaments immediately outside the disk.

The effects of substituting acetone for ethanol in the processing of the tissue were also explored. Some consequences of acetone dehydration are well recognized, e.g. a more clearly defined trilaminar structure in cytoplasmic membranes, and this was
observed, but no significant changes could be detected in the nature or arrangement of the Z-disk components.

**Tissue fixed in glutaraldehyde by method (ii): dehydration in ethanol**

All tissue components were poorly defined in this material despite prolonged heavy-metal staining, a consequence of the omission of the post-fixation treatment with osmium tetroxide usually given to tissues primarily fixed in aldehydes. This made analysis of the Z-disk structure very difficult, but such structure as could be seen in transverse sections appeared to be similar to that recorded following fixation by method (i).

**Tissue fixed with osmium tetroxide by method (iii): dehydration in ethanol**

In this material, as in that fixed in glutaraldehyde, longitudinal sections passing through the Z-disk presented a range of differing appearances, among which a few recurrent patterns could be distinguished. In some instances the I-filaments from adjacent sarcomeres appeared to be in longitudinal continuity across the disk, in others each I-filament appeared to fork into 2 branches which passed obliquely through the thickness of the disk to join the tip, and thus to form a component branch, of one of the I-filaments from the next sarcomere. The general visual effect produced by an array of such linking subfilaments is of a zig-zag pattern running down the disk and conforms to previous descriptions, for example, Knappeis & Carlsem (1962) (Figs. 7, 8). Less frequently the tips of the I-filaments of adjacent sarcomeres were seen to interdigitate, and there was a similar variation in the degree of overlap of the ends of the filaments between the small mitochondria-rich and the large mitochondria-poor fibres as has been described in glutaraldehyde-fixed material. This appearance of interdigitation and overlap of the ends of the I-filaments was most frequently seen in Z-disks in situations where fixation could be presumed to have been most rapid, that is, at the surface of fibres lying at the edge of the excised portion of tissue.

In transverse sections in which the disk was seen en face it usually possessed the ‘basket weave’ appearance described by Reedy (1964), being composed of a lattice of filaments approximately 5 nm thick, the sides of the enclosed rectangles being 20–25 nm (Fig. 9). In micrographs of slightly oblique sections the principal axes of this woven lattice could be seen to be disposed at 45° to the planes of lateral alignment of the I-filaments prior to their entry into the disk (Fig. 10). In a small number of disks the arrangement of filaments gave rise to a small 11-nm square lattice similar to that described to follow glutaraldehyde fixation. This fine square lattice was never so clearly defined as that which followed glutaraldehyde fixation and appeared predominantly in those Z-disks which might have been expected to have been fixed soonest, i.e. at the surface of fibres at the edge of the excised portion of tissue, and then most usually in small mitochondria-rich fibres, or in nuclear chain intrafusal fibres (Fig. 11).

Substitution of acetone for ethanol in the dehydration of osmium-fixed muscle caused almost complete loss of organized structure in the Z-disks, this effect being most obvious in transverse sections.
Fine structure of the rat Z-disk

The influence upon the structure of the Z-disks of post-mortem delay prior to fixation; glutaraldehyde fixation followed by osmium tetroxide, method (iv)

A short delay in fixation, up to 3 min, produced no demonstrable effect upon the fine structure of the Z-disk. After longer periods, 10 and 20 min, a slight deterioration in the definition of the lattice structure could be detected, but the form and spacing of the lattice never resembled the Z-disk structure normally seen to follow primary fixation with osmium tetroxide.

DISCUSSION

A number of studies of the fine structure of the Z-disk of vertebrate striated muscle have been published in recent years, and several of the authors have proposed models of its structure. Knappeis & Carlsen (1962) interpreted their observations upon osmium tetroxide-fixed amphibian muscle as showing the attachment of the end of each I-(actin) filament to 4, finer Z-filaments which passed diagonally through the thickness of the Z-disk, each to form one of the 4 similar filaments attached to the tip of each of the 4 adjacent I-filaments of the next sarcomere. The observations reported by Franzini-Armstrong & Porter (1964a) on the muscles of representatives of a number of orders were essentially similar, but they considered that the electron-dense Z material took the form of a membrane, pulled in opposite directions by the attached tips of the I-filaments from the adjacent sarcomeres, the consequent deformation of this membrane producing thickened ridges which corresponded to the electron-dense lines seen by Knappeis & Carlsen (1962) and interpreted by them as filaments. Reedy (1964) demonstrated a 'basket weave' appearance in transverse sections of the Z-disks of osmium-fixed rat striated muscle, and he accounted for this by a modification of the Knappeis & Carlsen model in which the 'weave' was provided by the tangential engagement of the Z-filaments with the tips of the I-filaments, the I-filaments themselves being 4-stranded helices having the same handedness on the two sides of the Z-disk. Most recently Kelly (1967) reports that with the aid of stereo electron micrographs it is possible to detect looping of the I-filaments, or of I-filament components, within the Z-disk, such that a component of one I-filament loops around the tip of a nearby filament of the next sarcomere, and then returns to form part of an I-filament in its own sarcomere adjacent to that from which it arose. This arrangement results in an interlocking square mesh of filaments each representing either half of the double-stranded, right-handed actin helix which forms the major component of the I-filament (Hanson & Lowy, 1963; Huxley, 1963; Depue & Rice, 1965), or one of the two tropomyosin B filaments which Hanson & Lowy (1964) have suggested may lie within the grooves of the actin double helix.

Franzini-Armstrong & Porter (1964a) have drawn an analogy between the dense material of the Z-disk and the cytoplasmic density associated with epithelial desmosomes, and Heuson-Stiennon (1965) has reported that primordial Z material in developing myoblasts is derived from the cytoplasmic density associated with 'coated' vesicles, originally part of cell contact points and later liberated into the cytoplasm, the
myofilament bundles finally coming to lie between these ‘Z-bodies’. However, not all workers who have examined the ultrastructure of myogenesis would wholly agree with this sequence of events (Allen & Pepe, 1965; Dessouky & Hibbs, 1965; Fischman, 1967). Kelly (1967) on the basis of these reports and on his own observations (Kelly, 1966) on the looping of the tonofilaments in epithelial desmosomes, suggests that there is a strong similarity between the looping filament configurations seen at these 2 sites, and he proposes that this may constitute a basic feature of both filament-to-membrane and filament-to-filament attachment systems.

All the workers who have made a specific study of the Z-disk agree upon its general appearance in transverse sections, namely, that of a square or woven lattice with a mean repeat distance of rather more than 20 nm, formed from fine filaments approximately 5 nm thick, each possibly composed of two 2-5-nm subfilaments (Huxley, 1963; Gonatas, 1966). Huxley (1963) has drawn attention to the similarity of this lattice structure to that seen in electron micrographs of tropomyosin B crystals, either sectioned (Hodge, 1959), or negatively stained (Huxley, 1963). Since then this likeness has been generally accepted to be an indication that tropomyosin B forms a significant component of the Z-disk structure (Hanson & Lowy, 1965).

In these connexions the observations presently described appear to be of some interest. It has been shown that when osmium tetroxide is used as a primary fixative for rat striated muscle, the fine structure subsequently observed conforms to the descriptions provided by previous authors; but that fixation with glutaraldehyde produces a new fine-structural pattern in both principal planes of section. Seen en face the disk shows a square lattice with a repeat distance of 11 nm, half that seen after osmium fixation, the axes of the lattice coinciding with the axes of alignment of the files of I-filaments immediately adjacent to the disk, rather than offset at 45°. Oblique linkages between the ends of I-filaments of contiguous sarcomeres have not been demonstrated in longitudinal sections, instead they appear either to interdigitate, or to be longitudinally continuous, with the filaments of the next sarcomere; no looping configurations have been observed.

There are four previously published reports in which illustrations of Z-disks appear to show this fine (11-nm) lattice morphology, and in all except one of these studies the fixative used was glutaraldehyde. The first is a picture by Uehara (1966) shown without further comment as a transverse section of a rat intrafusal muscle fibre. The second is in a paper by Gonatas (1966) on nemaline myopathy in which there are 3 illustrations (figs. 6, 9 & 10) of the normal Z-disk apparently showing the fine lattice in transverse sections; he considers these appearances, however, to be compatible with the original descriptions of Knappeis & Carlsen (1962) and of Franzini-Armstrong & Porter (1964a). The third example is in another paper dealing with nemaline myopathy by Engel & Gomez (1967). They illustrate (fig. 17) a normal Z-disk in transverse section, the centre of which shows the woven pattern of Reedy (1964), but the lower edge the fine (11-nm) lattice already described. It would not be appropriate to discuss here the details of the fine structure of the nemaline bodies but it is of interest that if the present observations upon the effects of fixation on the normal structure of the Z-disk are accepted, the appearance of transverse sections of nemaline bodies is very
The fine structure of the rat Z-disk

much more like that of the normal glutaraldehyde-fixed Z-disk than the last authors were prepared to accept; and the observation of Resnik, King-Engel & Nelson (1968) that hypertrophy and irregularity of Z-disks similar to that seen in nemaline myopathy can be found in the soleus muscles of cats 8-10 weeks after tenotomy, suggests that changes in Z-disk structure may not be the primary disorder in this disease, but rather a secondary response to some other disturbance of functional activity.

The remaining example is in Page’s (1965) study of frog twitch and slow muscle fibres in which an illustration of a transverse section of the Z-disk of a twitch fibre appears to show the fine-lattice pattern. The fixation here was with osmium tetroxide and this, therefore, is an exception to the correlation between fixative and fine structure which has been suggested above, but confirms the observations reported here that a small proportion of Z-disks do show the fine lattice where optimal conditions exist for fixation with osmium tetroxide.

The structure of the Z-line in insect muscle seems not to be directly comparable with that of vertebrates. Ashurst (1967), who has examined the Z-line structure of the flight muscle of belostomatid water bugs, found that the hexagonal spacing of the I-filaments in the I-band was preserved in the Z-disk where they interdigitate with those from the adjacent sarcomere. There was no evidence of I-filament splitting or of Z-filament formation and she concluded that the I-filaments were held in position in the disk by the amorphous Z-band material which lay between them. This Z-band material was so distributed that each hexagon composed of alternate filaments from the 2 sarcomeres surrounded a less dense ‘hole’, so that the disk appeared to be composed of a regular hexagonal lattice. These observations conflict to some extent with those of Shafiq (1963) who demonstrated a square lattice pattern (of approximately 25-nm period) in transverse sections of Z-disks of the indirect flight muscles of Drosophila melanogaster. However, Shafiq used osmium tetroxide as his fixative whereas Ashurst used glutaraldehyde, suggesting that in insect muscle also the choice of fixative may, to some extent, determine the fine structure of the Z-disk.

Although the final morphology of rat striated muscle appeared to be determined by the nature of the fixative, consideration was given to the possibility that other factors could significantly influence the final fine structure observed in the microscope. It seemed unnecessary to search for inter-specific differences of morphological response to different fixatives, since both forms of fine structure could be demonstrated within the same muscle, but it remained a possibility that there could be fine-structural differences between the 2 types of striated muscle fibres found in Mammalia, i.e. the small mitochondria-rich fibres and the larger diameter, relatively mitochondria-poor fibres; differences analogous to those described by Page (1965) between the Z-disk structures of the frog ‘twitch’ and ‘slow’ muscle fibres. The 2 types of fibre present in rat intercostal muscle were, in fact, distinguishable following glutaraldehyde fixation; there was a consistent increase in the extent of overlap of the I-filaments in the small mitochondria-rich fibres, and this was reflected in an enhanced contrast of the lattice pattern as seen in transverse sections.

Substitution of acetone for ethanol in the dehydration procedure leading to embedment in ‘Araldite’ had little fundamental effect upon the fine structure of
D. N. Landon

glutaraldehyde-fixed tissue other than a general loss of clarity; however, with osmium tetroxide fixation it proved markedly inferior to ethanol, almost all organized structure being lost from the Z-disk.

The effects upon fine structure of delays of up to 20 min between removal of the tissue and fixation were also explored. Increasing delay prior to fixation with glutaraldehyde caused a progressive loss of contrast in the final image of the Z-disk, but the lattice pattern was unchanged. However, using osmium-tetroxide fixation, there appeared to be some evidence of a change in the Z-disk structure with time, from the fine (1 nm) lattice to the larger woven form, but even where the fixation with osmium tetroxide was apparently most rapid, examples of the fine lattice were relatively infrequent and restricted to small mitochondria-rich fibres and ‘nuclear chain’ intrafusal fibres.

These observations do not permit any conclusions with regard to which of the 2 fixatives comes the nearest to fixation of the in vivo state, but such indications as there are appear to favour glutaraldehyde. Previous investigators of the effects of glutaraldehyde and osmium tetroxide upon other tissues, for example, Webster & Collins (1964), have in general preferred the former, particularly with respect to its effects upon membrane systems; the greatly improved preservation of the transverse tubular systems and the sarcoplasmic reticulum of striated muscle providing a good example (Franzini-Armstrong & Porter, 1964b; Page, 1965). Sabatini, Bensch & Barret (1963) have demonstrated that glutaraldehyde has a much less destructive effect upon tissue enzyme activities than has osmium tetroxide in the concentrations and time normally used for fixation, and Richards & Knowles (1968) have shown that very little difference can be detected between the X-ray diffraction patterns obtained from certain protein crystals before and after treatment with glutaraldehyde.

Such direct evidence as exists regarding the mode of action of glutaraldehyde suggests that the aldehyde groups react with side-chain amino groups of proteins and provide cross links between them (Johnston & Roots, 1967), an action which might be expected to be particularly effective within the Z-disk. This effect has not been demonstrated with osmium tetroxide however, its principal proven reactions being with the double bonds of unsaturated lipids (Adams, 1960; Aziz Khan, Riemersma & Booij, 1961), the site of this reaction not necessarily corresponding to the site of final deposition of osmium within the tissue (Riemersma & Booij, 1962). Elbers (1964) has suggested that the process of dehydration may reduce further dissolved and unreacted osmium tetroxide, the resulting lower oxides accumulating near hydrophilic groups within the tissue. Hopwood (1969a, b) has recently compared the effects of a number of fixatives commonly used in electron microscopy upon the electrophoretic mobility and gel-filtration characteristics of a variety of proteins. He found that glutaraldehyde was the most efficient cross-linking agent of all the fixatives investigated, the majority of proteins being homopolymerized to such an extent that they were unable to enter the gel. Osmium tetroxide, on the other hand, was found to increase the electrophoretic mobility of proteins and was at the same time bound to them.

A further hint that glutaraldehyde may yield the ‘best’ state of preservation of the Z-disk is provided by the observation reported above that where the osmium tetroxide
fixative might be expected to act most rapidly, and is in highest concentration, the final appearance of the Z-disk approaches that of the aldehyde-fixed material.

The fine-structural features of the Z-disk reported here to follow glutaraldehyde fixation are difficult to reconcile with the earlier model structures proposed by Knappeis & Carlsen (1962), Franzini-Armstrong & Porter (1964) and Reedy (1964), but they do appear to be compatible with a modification of the looping filament model proposed by Kelly (1967). This is envisaged as being composed of 2 overlapping square lattices, each of approximately 22-nm period, out of register one with another by 50% along each axis, and formed from the looping or cross-linking of the ends of the interdigitating I-filaments with the ends of immediately adjacent I-filaments from their own sarcomere.

The transformation of this glutaraldehyde-fixed structure to that seen following osmium tetroxide fixation could be brought about by a realignment of the fine cross-linking filaments, such that they are no longer connected to the I-filaments of the same sarcomere but rotate through 45° in a transverse plane and link with the tips of the I-filaments of the adjacent sarcomere. This process would have the effect of ‘unlocking’ the 2 lattices, allowing the interdigitating I-filaments to slide apart until tethered by the linking filaments. In their new position they would correspond to the Z-filaments of Knappeis & Carlsen (1962), to give a narrow zig-zag Z-disk seen in longitudinal sections following osmium tetroxide fixation, and the large single lattice in transverse sections, its axes set at 45° to the direction of alignment of the files of I-filaments in the I-band adjacent to the disk. Although a wire model has been constructed which in many respects simulates these structural arrangements in terms of looping I-filament components, on the lines of Kelly’s (1967) models, it does little more than define the observed fine structure in diagrammatic form, the chemical nature and macromolecular configurations of the moieties involved remaining obscure. It does not indeed fully describe the observed structure since it is apparent that the linkage of the adjacent I-filaments is not a simple looping from the end of one filament to the next, but is a more extensive lateral attachment extending throughout most of the zone of overlap, similar in many respects to that suggested by Ashurst (1967) to occur between the adjacent I-filaments in the Z-disks of insect muscle.

As noted earlier, Huxley (1963) has drawn attention to the similarity between the appearance of the transversely sectioned Z-disk and the lattice of tropomyosin B, and this similarity has subsequently been taken to indicate that tropomyosin B makes a substantial contribution to Z-disk structure. The observations reported here do not in themselves conflict with such an hypothesis, they merely require that a second tropomyosin B crystal be superimposed upon the first, each being connected to the set of I-filaments arising from one sarcomere. To date, however, other lines of research have yielded little concrete evidence in support of such a localization of tropomyosin B within the Z-disk. Pepe (1966), using immunological methods, failed to identify tropomyosin B at the Z-line in chicken breast muscle. Stromer, Hartshorne & Rice (1967) have shown that treatment of rabbit psoas muscle with a dithiothreitol solution removes the dense material at the Z-disk, and that this can be replaced in large part by allowing the previously extracted muscle to interact with certain
fractions of the extract; the most effective fraction in this respect being deficient in tropomyosin B. On the other hand Rash, Shay & Biesele (1968) have achieved extraction of Z-bands from chicken muscle with concentrations of urea which appear to leave other elements of the sarcomere, actin, myosin and M-line protein, undisturbed, and they consider that the tropomyosins are the only other structural proteins present in sufficient amounts to account for the extracted Z-bands. Masaki, Endo & Ebashi (1968) have demonstrated the exclusive localization of another protein, the 6-s component of L actinin, to the Z-bands in chicken muscle, and Briskey, Seraydarian & Mommaerts (1967) have suggested that L actinin may fulfil a structural role within the Z-disk in view of its high cross-linking affinity for actin. Masaki et al. (1968) point out, however, that the 6-s L actinin component does not exceed 3% of the total muscle protein and that the Z-disk must, therefore, contain some other protein moiety; they suggest that this is most likely to be tropomyosin. It is apparent that the chemical characterization of the Z-disk awaits further biochemical analysis, and that attempts to identify its components from morphological evidence could well prove misleading.

The demonstration of 2 alternative fine-structural patterns for the Z-disk material, dependent upon the manner of fixation, finally raises the possibility that configurational change between the 2 forms may have a role in the processes of muscular contraction. Elliott, Lowy & Millman (1967) have examined the changes in the X-ray diffraction patterns from living toad sartorius muscle which accompany contraction. They obtained faint equatorial reflexions, at all muscle lengths, which they have tentatively identified as being derived from the square lattice of I-filaments immediately adjacent to the Z-disk, and they find that the spacing of these reflexions changes from 19.7 to 30 nm between the resting and contracted state. They suggest that this I-band lattice may undergo an expansion during contraction similar to that which they have demonstrated to occur in the actin/myosin hexagonal lattice, and they quote a personal communication from M. K. Reedy of electron-microscopic observations that the spacing of the Z-line and adjacent I-band lattices of frog sartorius muscle vary inversely with sarcomere length from between 20 to 30 nm. If tropomyosin should prove to be a major component of the Z-disk the observations of Cohen & Longley (1966) suggest an explanation for the mechanism underlying such changes in lattice spacing. They found that isolated tropomyosin paracrystals show a polymorphism which is dependent upon both divalent cation concentration and pH; above a critical divalent cation concentration fibres with a large period (40 nm) are formed, below that concentration (near the isoelectric point), crystallization occurs into lattice structures with a reduced period. They suggested that reversible binding of divalent cations by tropomyosin might occur in vivo, and that this process might have a regulatory function in muscular activity.

Such observations provide some of the basis for the theory of contraction of striated muscle recently put forward by Ullrick (1967), in which he proposes that the Z-disks undergo expansion with activation to exert a force perpendicular to the sarcolemma. Due to the cohesive properties of the sarcoplasm such an outward tension would result in an inward tension parallel to the long axis of the fibre, with, if permitted, shortening and widening of the whole fibre. The sarcolemma would play no role in
transmitting tension to the ends of the fibre, but serve as a container for a constant volume of sarcoplasm and act as a barrier to the exchange of fluid across the fibre surface. He considers that the resting tension and resting length of muscle are set by (a) a weak bonding between the thick and thin myofilaments, the bonding being broken by activation, and (b) by the elasticity of the sarcolemma. He claims that these propositions are consistent with the sliding-filament morphology and do not require elaborate interacting mechanisms between the actin and the myosin.

Conversion of the fine (11-nm) lattice to the larger form by the method suggested earlier would cause an increase in the area of the Z-disk of approximately 30%, provided the linking filaments remained of constant length and at a constant inclination to the long axis of the fibre. For the volume of the fibre to remain constant such an increase would entail a reduction in the length of the adjacent sarcomeres by about 45%, a figure compatible with the shortening observed during the contraction of living muscle (Huxley & Niedergerke, 1954). Therefore, one possible interpretation of the observations reported in this paper would be that they may provide some morphological support for the somewhat heterodox concept of the mechanism of muscular contraction proposed by Ullrick (1967). However, a large body of evidence exists, gained from a variety of different experimental approaches, which supports the current hypothesis that the actomyosin system is the energy transducer in skeletal muscle. It would appear most likely that if the configurational changes observed in the Z-disk do reflect differences between resting and active muscle, these changes are the consequence of the interactions occurring between the actin and myosin filaments during contraction, rather than evidence of the generation of the contractile force within the disk.

The author wishes to express his gratitude to Professor J. B. Cavanagh for his constant encouragement and advice and to Professor H. E. Huxley for his helpful criticisms of the manuscript; to Mrs A. P. Lavery for her work on the typescript and Mr I. Saxton for technical assistance; and to the Medical Research Council and the Multiple Sclerosis Society of Great Britain and Northern Ireland for providing the necessary facilities for electron microscopy.

REFERENCES


Fine structure of the rat Z-disk


(Received 6 February 1969—Revised 5 June 1969)
Fig. 1. A longitudinal section of a small mitochondria-rich fibre. Interdigitation of the terminal portions of the I-filaments from adjacent sarcomeres is evident within the thickness of the disk, with increase in their density throughout the zone of overlap, and extending for a short distance into the I-band. Glutaraldehyde and osmium tetroxide. ×100,000.

Fig. 2. A longitudinal section through the Z-disk of a similar fibre. Here the orientation of the Z-disk components is such that the I-filaments from the adjacent sarcomeres appear to be in longitudinal continuity. Glutaraldehyde and osmium tetroxide. ×100,000.

Figs. 3, 4. The Z-disk of a small mitochondria-rich fibre (Fig. 3) for comparison with a disk from an immediately adjacent large mitochondria-poor fibre in the same section (Fig. 4). The I-filament overlap in the former is 60–70 nm, in the latter, 20–30 nm. Glutaraldehyde and osmium tetroxide. ×100,000.
Fine structure of the rat Z-disk
Fig. 5. A slightly oblique transverse section of a Z-disk demonstrating the 11-nm square-lattice structure which has been observed to follow glutaraldehyde fixation, and also the presence within the one disk of several smaller areas of the lattice with their axes at angles to one another. Glutaraldehyde and osmium tetroxide. × 100,000.

Fig. 6. A transverse section at a small angle to the plane of the Z-disk showing the square array of I-filaments immediately adjacent to the disk (arrows), and the alignment of this array with alternate bands of the fine 11-nm lattice of the disk proper. Glutaraldehyde and osmium tetroxide. × 100,000.
Fine structure of the rat Z-disk
Fig. 7. A longitudinal section of the Z-disk of a large mitochondria-poor fibre fixed solely in osmium tetroxide. A number of the I-filaments appear to fork at their tips, each branch being continuous with an I-filament from the adjacent sarcomere. Osmium tetroxide. \( \times 100,000 \).

Fig. 8. A longitudinal section of a small mitochondria-rich fibre. The Z-disk is thicker and a criss-cross arrangement is visible at its centre, apparently produced by oblique linkages between the ends of the I-filaments of the adjacent sarcomeres. Osmium tetroxide. \( \times 100,000 \).

Fig. 9. A transverse section of a Z-disk fixed solely in osmium tetroxide. The 'basket weave' lattice described by Reedy (1964) is clearly visible, the dimensions of each rectangle being approximately 20 × 25 nm. Osmium tetroxide. \( \times 100,000 \).

Fig. 10. A transverse section at a small angle to the plane of the Z-disk in a fibre similarly fixed. The axes of lateral alignment of the square array of I-filaments immediately adjacent to the disk (small arrows), can be seen to be placed at 45° to the axes of the woven lattice of the Z-disk itself (large arrow). Osmium tetroxide. \( \times 100,000 \).
Fine structure of the rat Z-disk
Fig. 11. A transverse section through a Z-disk of a fibril at the edge of a tissue sample fixed in osmium tetroxide, a site where fixation is presumed to have been most rapid. Whereas there is some suggestion of a woven appearance to the left centre (asterisk), the lower margin of the disk (arrow) more nearly approaches the structure normally seen to follow aldehyde fixation. Osmium tetroxide. × 100000.