ELECTRON MICROSCOPE STUDIES OF MUSCLE STRUCTURE

by

R. REED AND K. M. RUDALL

Department of Biomolecular Structure, The University, Leeds (England)

The methods of layer-stripping, metal-shadowing and layer-digestion[1] enable us to study in the electron microscope details of organisation extending over a large area. The aim of such studies is to observe structure at any level in a tissue or cell, and particularly the interrelationships of elements of structure. To date, successful analyses of muscle structure using the electron microscope have been made on soluble products — myosin[2, 3], actin[4, 5] and tropomyosin[6]; and on teased fragments (myofibrils) of smooth[7] and striated muscles[3]. To add to these valuable contributions we describe below methods of observing levels of structure in whole muscle cells as distinct from fragments of cells.

The materials used in our experiments have been leg muscles from the frog and toad, intestinal muscles from the frog, and smooth muscles from the molluscs *Mytilus edulis* and *Mya arenaria*. While for some purposes no fixative was resorted to, in general the muscles were fixed in 5 % formaldehyde for 24 hours.

Layer-stripping. Fixed muscles were split by a needle or scalpel into ribbons ½—1 mm thick, then placed on a glass slide and allowed to dry in a desiccator over P_2O_5. When dry the muscle layer was lifted at a corner and torn away gently from the slide with forceps. As a result a thin layer* of the muscle tissue remained adhering to the slide. This material consisted of whole cells, or parts of cells with various levels of the cell structure exposed, or regions of the connective tissue. The newly exposed surface was shadowed with gold[8] and then coated with a thin layer of collodion.

Layer-digestion. The slide was placed in a bath of pepsin/hydrochloric acid and digested for 24 hours at 40° C. After washing, the slide was transferred to a trypsin bath at p_H 8.6 and digested for a further 24 hours. The result of these digestions was to remove the formalin-fixed tissue (or unfixed tissue), while leaving the gold shadow-cast of the muscle surface adhering to the collodion. After washing in water the film was ready for observation in the electron microscope. All the photographs were taken using the R.C.A. Type B instrument and a 45 kV electron beam.

STRUCTURE OF STRIATED MUSCLE TISSUE

In the present paper we describe results obtained with striated muscle tissue only. In the first instance unfixed muscle was used, and though the results were of interest there were obvious complications owing to autolysis during drying. Certain results obtained by extracting some of the muscle constituents or by treating in trypsin in order to facilitate study of the collagen fraction served at least to indicate the considerable range of experimental possibilities opened up. At present we describe only the main

* This may also be examined in the electron microscope directly or after "staining".

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structural features observed in formalin-fixed material, which gave better defined results than unfixed material did. Indeed, it would seem valuable to follow any preliminary extraction of a given constituent by appropriate fixation.

In frog leg muscle there are numerous structures which we may expect to see — connective tissue elements, blood vessels, nerves, sarcolemma, motor end plate, nuclei, myofibrils, etc. With the many levels exposed by layer-stripping probably all these structures will be seen at one time or another. In practice, the levels most frequently seen relate to the connective tissues, the surface of the intact muscle fibre, the under surface of the sarcolemma, and various surfaces of the myofibrils, external and internal. We describe below some structural features of these principal levels.

The surface of the muscle fibre. In this work no attempt has been made to obtain muscles in a state of contraction. The muscles were fixed in formalin while either held taut or stretched by about 20%. The surface of a muscle fibre (Fig. 1) shows two main features, the fine regular fibrils on the surface, and the undulations due to the muscle striations. The fine fibrils comprise several layers between the muscle fibres, and many views of these networks unattached to muscle fibres have been obtained. Similarly, different muscle fibres when viewed as in Fig. 1 have coverings of fibrils of few or many layers according to where the cleavage occurred between the fibres. These fine fibrils are comparatively uniform in diameter (cf.[3]) and are banded like connective tissue collagen[9]. They often occur in pairs and many run in a closely parallel course. In Fig. 1 the parallel system makes an acute angle with the axis of the muscle fibre as if following a spiral course round the fibre cylinder. In different preparations the fibrils appear in all aspects from closely parallel and straight or sinusoidal courses to irregular networks. Fibrils such as those in Fig. 1 are so closely adhering to the surface of the muscle fibre that they must contribute to the structure of the sarcolemma. However, they could be either of fibroblast origin, or they could be produced from the muscle cell in a manner not unlike that of the collagen-type fibrils produced by the epidermal cells of earthworms[1]. What we have to decide is whether there is an additional true sarcolemma between the fibril network and the muscle cell substance. This point is considered below.

In Fig. 1 the position of the muscle bands is indicated by the broad undulations. All the photographs are printed as negatives, which means that ascending slopes (with reference to the direction of metal-shadowing) appear light and descending slopes dark.

The surface of the myofibrils. At some regions, as in Fig. 2, the sarcolemma is torn away revealing the surface of the myofibrils. The final identification of the bands will depend on optical microscope studies of wet fibres, dry fibres and the same when shadowed with gold. The provisional interpretation of the A (anisotropic) and I (isotropic) bands is indicated in Fig. 2, and is based on the observation that in stretched specimens (Fig. 3) the I band is increased in width. The most striking feature of the A bands is that they appear raised up above the level of the I bands; and each A band is divided into two halves separated by a depression marking the position of the mesophragmata. We cannot assess as yet the difference in height of the A and I bands and still less its relation to the total thickness of the myofibril. However, we may conclude that there is more solid matter in the A band than in the I band, and a greater amount of water in the I band of fresh muscle. The edges of the A band appear to make a sharp descent to the level of the I bands. In the middle of the I bands there are slightly raised levels which again appear to be double, particularly in Fig. 3, with a depression at the position of the Z band. Many of the features of Figs. 2 and 3 undoubtedly constitute a view of

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Fig. 1. Surface of striated muscle fibre showing fine fibrils cross banded like collagen, and the undulations due to the muscles striations

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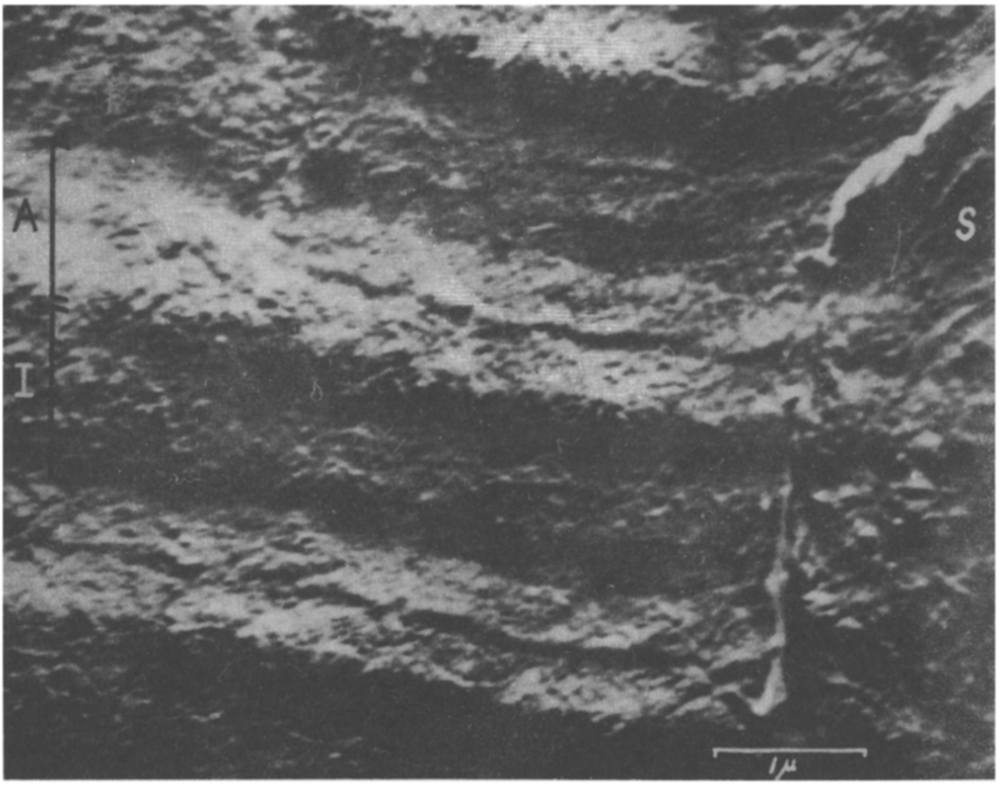

Fig. 2. Sarcolemma (S) torn away revealing the surface of the myofibrils

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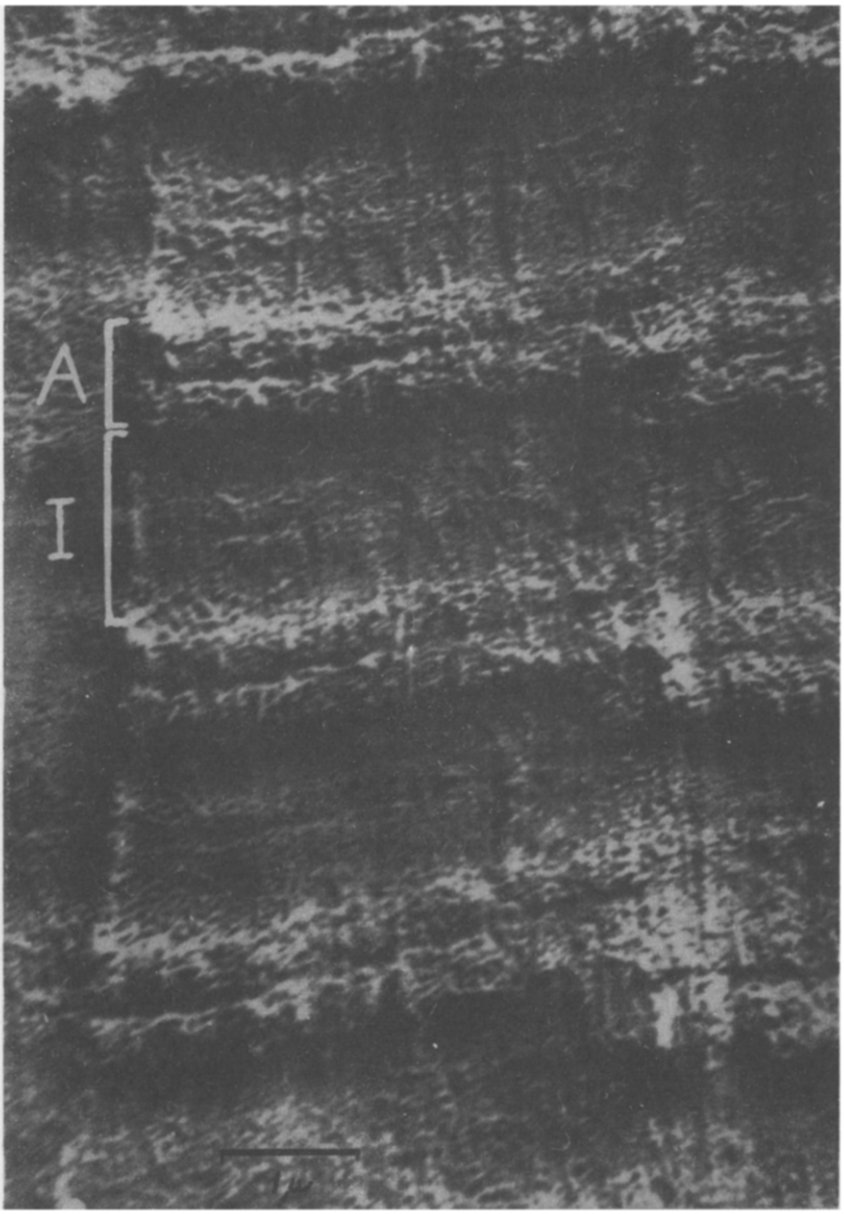

Fig. 3. Form of myofibrils from stretched muscle

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Fig. 4. Level in muscle between fibril layer (F) and the myofibrils. Shows regular, discrete corpuscles resting on an undulated membrane

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the muscle fibrils coated with sarcoplasm. If it should prove possible to remove the sarcoplasm before making the replicas, then gradually deeper levels in the structure of the myofibrils should be disclosed for examination.

In Fig. 3 there are three adjacent myofibrils which are slightly displaced relative to one another. This kind of longitudinal displacement and variations of it are very common. There is the curious appearance of the upper half of an A band running continuous with the lower half of the adjacent A band, a condition which may conceivably be a natural one.

This brief description of the myofibrils illustrates the kinds of observations to be made at various levels within the muscle structure. There are almost unlimited opportunities for further study, e.g., where the sarcoplasm is removed and the constituent proteins and other substances are selectively reduced or eliminated. Parallel with such an analysis of the distribution of components, studies could also be made on the different types of contraction.

THE SARCOLEMMA AND OTHER FEATURES

The outer surface of the sarcolemma may be represented by the background between the fibrils as illustrated in Fig. 1. Sometimes the fibrils have been largely removed in the layer-stripping process and the surface of the sarcolemma then appears as at S, Fig. 2 — a thin membrane overlying the myofibrils. This membrane does not show a predominantly fibrous structure. A few bodies which seem to be discrete corpuscles lie on its surface. Further studies will doubtless test the hypothesis that this membrane is the true sarcolemma.

In the same preparation that gave the structures illustrated in Figs. 1 and 2 we have observed the very striking structure shown in Fig. 4. The composition of this is as follows. At a lower level F we see the collagen-type fibrils which are characteristic of the external surface of the muscle fibre. Superposed on this network is an undulating membrane the edges of which are torn parallel to the axis of the muscle fibre, while the undulations can be recognised as related to the striations of the latter. Distributed over the surface of this membrane are corpuscles approximately 500 A in diameter and of comparatively uniform size. Such a picture may very well correspond to the internal surface of the true sarcolemma, i.e., the membrane illustrated at S in Fig. 2. Or it may correspond to any of the structures lying beneath the sarcolemma, e.g., nucleus or motor end plate; or it may be related to a natural stage of degeneration or to a pathological state of the myofibrils. It is included in this description of muscle as a further example of the numerous levels of structure which may be examined.

Most of the main features shown by gold shadow replicas, as prepared by layer-stripping and layer-digestion, are directly related to the structure of the dried tissue and should be fully interpretable with the aid of additional experimental work. But false views of details are obtained when the gold aggregates instead of remaining in the thin film as deposited. We have sought to minimise this and are developing other methods which may be without this defect.

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SUMMARY

1. The methods of layer-stripping, gold-shadowing and layer-digestion are applied in electron microscope studies of striated muscle. The methods enable us to observe structures and degrees of organisation that would be destroyed in procedures such as grinding and fragmentation.

2. The fine, uniform collagen-type fibrils of the connective tissue have been observed and also the way in which these form a close covering of the sarcolemma.

3. The surface of the myofibrils is described in different states of extension. The form of the presumed A band is such as to indicate that it contains more solid matter than the I band.

4. Various other levels of structure have been observed. Attention is drawn to the presence of regular discrete corpuscles which may be identifiable with enzymes or other important constituents of the muscle fibre.

RÉSUMÉ

1. Les méthodes d'enlèvement des couches, d'obtention de contrastes par l'or, et de digestion en couches, sont utilisées dans des études du muscle strié faites à l'aide du microscope électronique. Ces méthodes permettent d'observer des structures et des degrés d'organisation qui seraient détruits dans des procédés tels que le broyage ou la fragmentation.

2. Les minces fibrilles, uniformes, du type collagène, du tissu conjonctif, ont été observées, de même que la façon dont ces fibrilles constituent une enveloppe du Sarcolemma.

3. La surface des myofibrilles est décrite dans différents états d'extension. La forme de la bande A est telle qu'elle semble contenir plus de matière sèche que la bande I.

4. Différents autres niveaux de structure ont été observés. L'attention est attirée sur la présence de corpuscules réguliers bien différenciés, qui pourraient être identifiés avec des enzymes ou d'autres constituants importants de la fibre musculaire.

ZUSAMMENFASSUNG

1. Die Methoden der Schichtabstreifung, Goldschattierung und Schichtverzehrung werden bei elektronenmikroskopischen Untersuchungen des quergestreiften Muskels angewandt. Diese Methoden ermöglichen uns, Strukturen und Organisationsgrade wahrzunehmen, die bei anderen Verfahren, wie z.B. Feinreiben und Fragmentierung zerstört werden würden.

2. Die feinen, gleichförmigen, kollagenartigen Fibrillen des Bindegewebes wurden wahrgenommen und ebenfalls die Weise, auf die sie eine geschlossene Bedeckung des Sarcolemmas bilden.

3. Die Oberfläche der Myofibrillen wird bei verschiedenen Ausdehnungszuständen beschrieben. Die Form des angenommenen A-Bandes könnte andeuten, dass es mehr festen Stoff enthält als das I-Band.

4. Verschiedene andere Strukturniveaus wurden wahrgenommen. Es wird besonders auf das Vorhandensein von regelmässigen diskreten Körperchen hingewiesen, die mit Enzymen oder anderen wichtigen Bestandteilen der Muskelfaser identisch sein können.

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