The Effect of Tilting Ultrathin Sections on the Image of the Z-Disc of Skeletal Muscle

The fine structure of the Z-disc of skeletal muscle presents a variety of images in the electron microscope. Several interpretations have been advanced to account for this phenomenon and some tentative models have been proposed to explain the 3 dimensional structure of the disc[1–7]. However, these interpretations and models derive from examination of electron micrographs obtained without the aid of electronmicroscopes equipped with a goniometer specimen stage. In the present report we describe for the first time the effect of tilting the Z-disc in an electron microscope fitted with a goniometer stage capable of giving $\pm$ 60° of tilt. The results thus obtained are then compared with projected images of a three dimensional model of the Z-disc.

Materials and methods. Fragments of the sternomastoid muscle, freshly taken from a rat, were fixed in 4% glutaraldehyde at pH 7.4 and post-fixed in 1% osmium tetroxide. After dehydration in graded alcohols they were treated with propylene oxide and embedded in Epon. Ultrathin sections (silver to grey) cut both longitudinally and transversely to the Z-disc, were stained with lead citrate and uranyl acetate and examined with a Siemens Elmiskop 1, a Philips EM 300 and a Jeol JEM 100B electron microscope, the latter two being fitted with a goniometer stage and a tilting specimen holder. A model of the Z-disc, based on the work of KNAPPEIS and CARLSEN[1] and our own observations, was constructed from perspex rod (Figure 1) and its shadow was projected

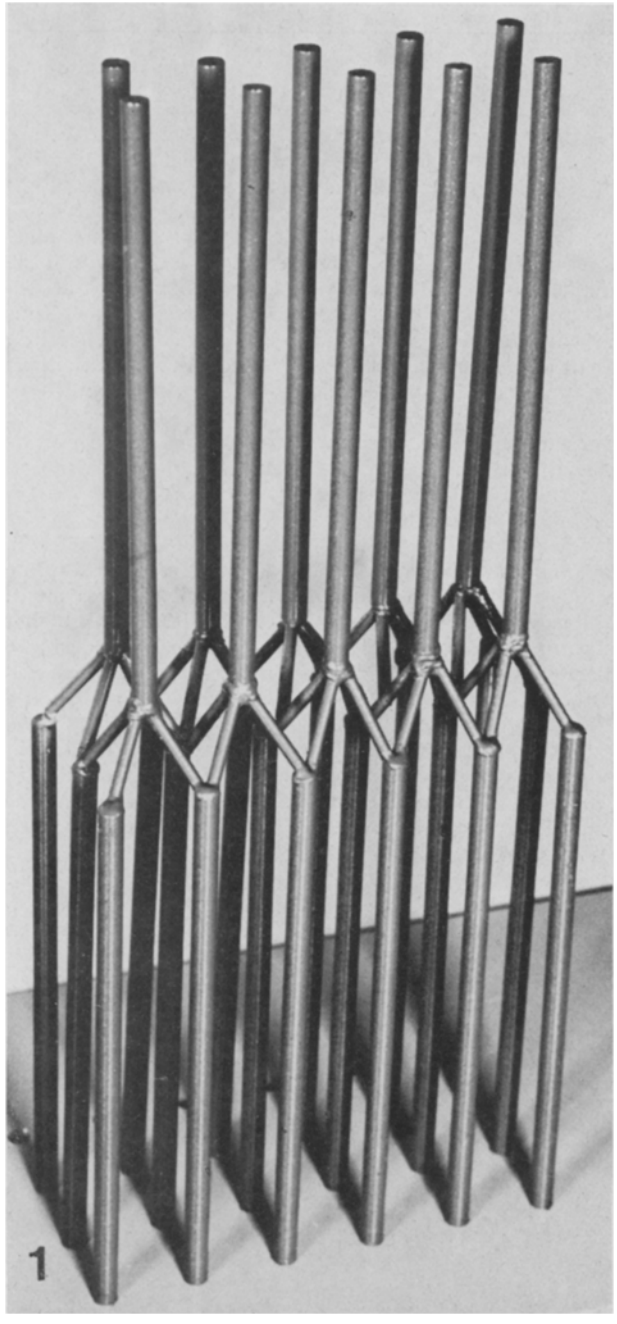

Fig. 1. 3-dimensional model of the Z-disc used to interpret the images seen in the electron microscope.

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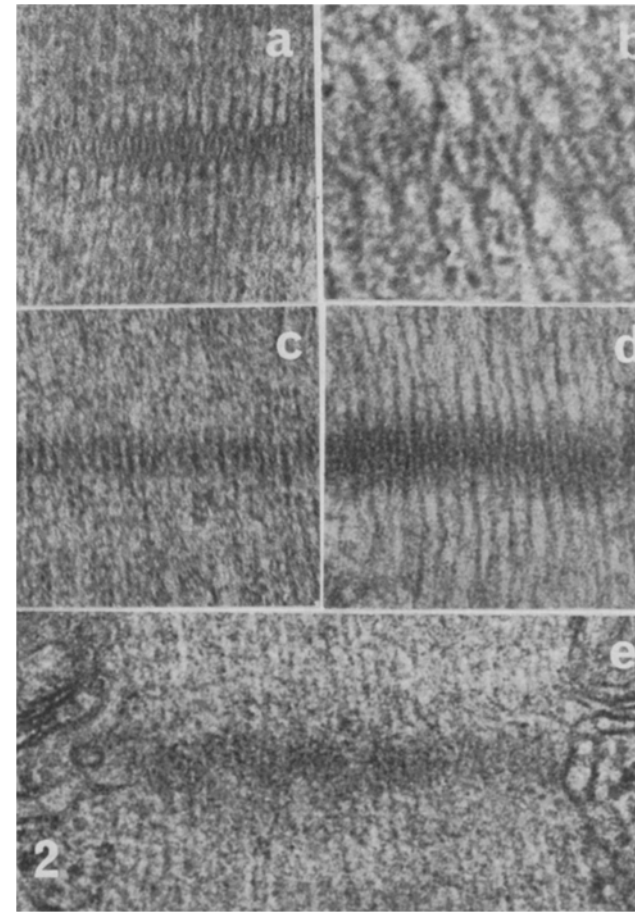

Fig. 2. Different images of the Z-disc of skeletal muscle observed in conventional electron micrographs (see text). a), c), d), e) × 98.000; b) × 240.000.

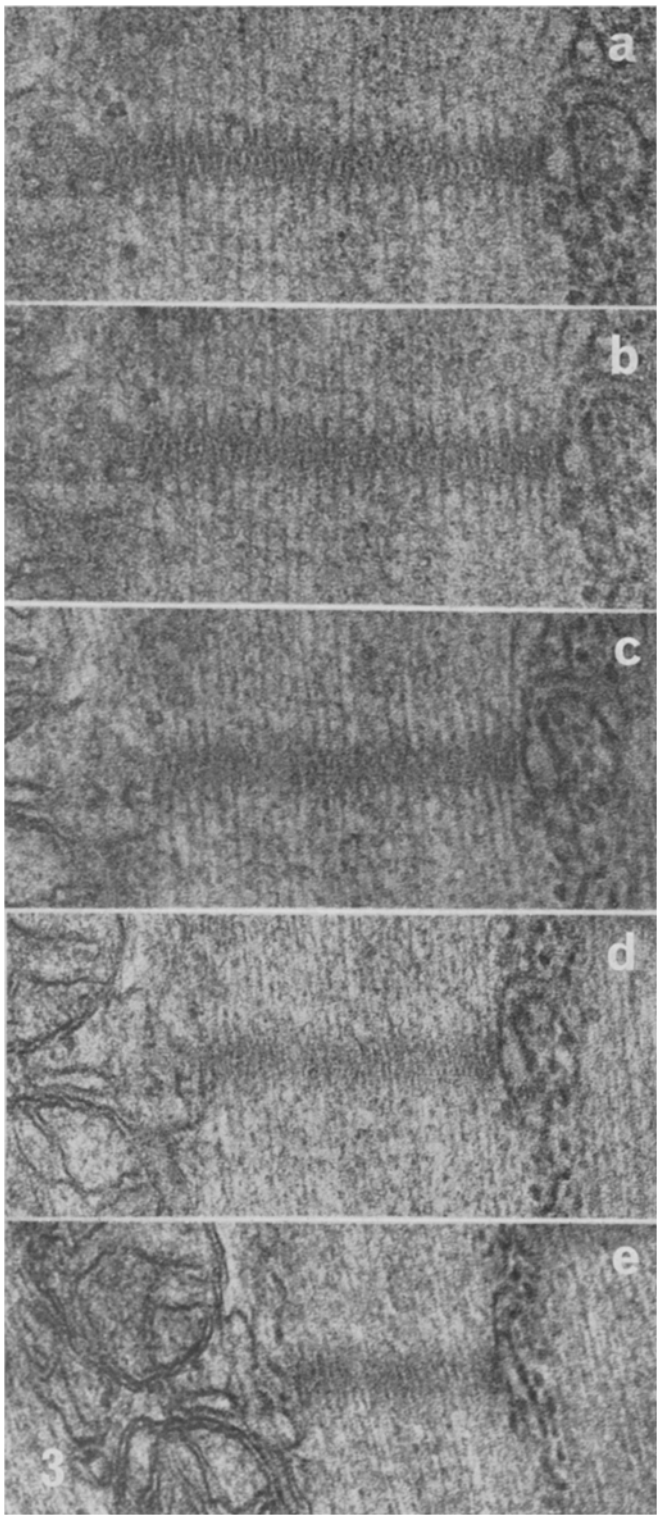

Fig. 3. a) to e) show the result of tilting (rotating a longitudinal section of a Z-disc about the long axis of the myofibrils. a), b), c), d) and e) represent 0°, 15°, 30°, 45°, and 60° of tilt respectively. × 90.000.

onto a screen. By changing the orientation of the model we were able to observe the changes in pattern of the projected shadow and to compare them with the changing images observed when tilting the ultrathin sections in the electron microscope.

Results and discussion. Four major patterns of the Z-disc were observed in conventional electron micrographs obtained without the aid of the goniometer-tilting device. Those we have called church steeple, straight line, interdigitating, and fuzz. The church steeple pattern is characterized by a single or a double zig-zag line with actin filaments attached to the apices (Figure 2a and b). In the straight line pattern the actin filaments seem to be continuous and to run in a straight line between adjacent I-bands and in the region of the Z-disc the filaments appear thicker (Figure 2c). In the inter-

digitating pattern straight actin filaments from adjacent I-bands appear to be discontinuous and to alternate in the region of the Z-disc (Figure 2d). The fuzz image is the most common and the Z-disc does not exhibit any clearly definable pattern and appears diffuse (Figure 2e). Tilting longitudinally oriented specimens (rotation of the specimen about the longitudinal axis of myofibrils) through the $\pm 60°$ obtainable with the electron microscope equipped with a goniometer stage revealed that considerable changes occur in the appearance of the Z-disc. With a starting image of a church steeple pattern (Figure 3a) a tilt of 15° produced little change apart from a less clear image (Figure 3b). With a tilt of 30° and 45° the disc was converted into a fuzz image (Figure 3c and d) which in turn emerged as a straight line at the maximum tilt of 60° (Figure 3e). Tilting the section through the 60° in the manner described above also produced a decrease in the width of the myofibrils, approximation of actin filaments, and an enhanced contrast of the specimen (Figure 3). Tilting the same Z-disc at right angles to the previous series showed that the initial image of a church steeple pattern gradually changed to the interdigitating pattern without any obvious intermediate fuzz stage (Figure 4). Tilting transversely cut sections of the myofibrils at the level of the Z-disc failed to show any significant alteration of the image no matter in what direction they were tilted.

By projecting the perspex model (Figure 1) representing 2, 3 or 4 rows of actin filaments we have found a high degree of correlation between the patterns produced by altering the orientation of the model and by tilting the specimens. However, the degree of tilting required to produce changes in pattern did not correspond precisely in the 2 systems.

The above results provide evidence for the first time that some of the varying images of the Z-disc that are seen with the electron microscope are accounted for by the orientation of the specimens. They also suggest that the three dimensional structure of the Z-disc is essentially similar to that proposed by KNAPPEIS and CARLSEN[1] but probably with a configuration less rigidly ordered than has been previously indicated. The results clearly show that the fuzzy appearance of the Z-disc, often interpreted as an indication of the presence of a diffuse matrix permeating the Z-disc filaments[10, 2] may be due to the orientation of the specimen which results in the non-alignment of the filaments. Our findings together with those of previous authors, however, offer no explanation for the unusually broad Z-line found in the cat myocardium[8] or in the cases of nemaline myopathy[9].

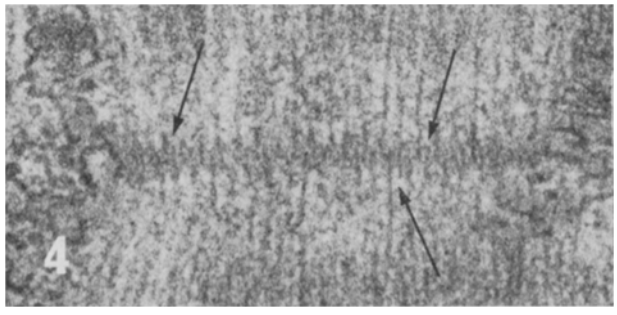

Fig. 4. The same Z-disc as in Figure 3, this time tilted (rotated) about its long axis, which is at right angles to the previous figure. Interdigitating filaments are seen particularly where arrowed. × 90.000.

Résumé. A l'aide du microscope électronique muni d'un goniomètre, la structure tri-dimensionnelle du disque Z de la fibre musculaire striée a été analysée. Les résultats obtenus sont comparés avec un modèle construit en perspex et avec les images conventionnelles du disque Z.

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Changes in the Differentiation of the Sea Urchin Larva by Action of a Detergent upon the Unsegmented Egg

The normal development of sea urchin eggs depends on the balance between two different trends governing respectively the differentiation of ectodermal and entomesodermal parts of the larvae. This balance can be displaced by treating the eggs with certain chemicals. In the animalization, for example, the differentiation of ectoderm is favoured at the expense of the entomesoderm. The mode of action of animalizing agents is not known. An action on the cell surface has been suggested[1-3].

Detergents represent an interesting class of chemicals able to react with the cell membrane, disturbing its structure and removing proteins fixed on the surface of membrane. Earlier observations with sea urchin eggs have shown interesting effects of detergents on the differentiation of larvae. An anionic detergent, lauryl sulfate, was shown to be able to produce radialization of larvae[4]. Radialization corresponds to a weak animalization. According to RUNNSTRÖM[5], this detergent increased the frequency of animalization in eggs pretreated before fertilization with thiocyanate. RUNNSTRÖM made the interesting observation that the detergent was most effective if the eggs were exposed to its action very early after fertilization. Chemicals with a high surface activity like salts of bile acids were shown to be effective animalizing agents[6].

In this paper we shall try to elucidate the mode of action of a detergent and to obtain information about the

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