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Freeze-Etching Studies on Muscle

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Freeze-etching studies on muscle

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[Plate 26]

The technique of freeze-etching is illustrated with reference to striated muscle. Besides features of immediate biological interest, the material demonstrates various ways in which the process may be used in general to yield new information. These fall broadly into two classes: (*a*) qualitative: visualizing structures not readily seen by other methods, for example, general three-dimensional structure (low resolution) and membrane particles (high resolution); (*b*) quantitative, for example, the distribution of membrane features over extensive uneven surfaces (low and high resolution).

1. Introduction

This communication describes an electron microscopic survey of vertebrate striated muscle with particular reference to fish body muscle as revealed by the method of freeze-etching using a standard Balzer's freeze-etch apparatus. Attention is drawn to the more interesting features which demonstrate the advantages and general applicability of this method of specimen preparation to biological materials. The pretreatment of the specimens was basically as described previously by Professor Moor and Dr Branton. In the description of replicas of membranes, images are referred to as 'outer' (as seen from the outside of the cell or organelle) or 'inner' (seen from within the cell or organelle) with no special reference or inference as to the specific plane of membrane fracture, a subject discussed earlier by Dr Branton.

2. Results

(*a*) *Earlier results*

Early freeze-etching work on mammalian heart muscle cells revealed a clear regular array of relatively large invaginations or transverse (T) tubule apertures in the cell membrane (Rayns, Simpson & Bertaud 1967, 1968*a*). This was followed up by a similar study on mammalian skeletal muscle, where broad transverse belts of numerous small depressions in the cell membrane were demonstrated, suggesting that there is no direct communication between the transverse tubule and the sarcolemma. This correlated well with thin section studies using lanthanum staining where a multiple channel indirect communication with the sarcolemma was demonstrated (Rayns, Simpson & Bertaud 1968*b*).

(*b*) *Recent results*

(i) *General*

Encouraged by these investigations, the freeze-etching technique was applied to the body muscle of a fish (Bertaud, Rayns & Simpson 1970). A general longitudinal fracture reveals details of a muscle cell and views of the extracellular material, see figure 1, plate 26. One can see the longitudinal array of thick filaments, F, and tubules of the triad, T, S, within the cell at the level of the Z-line, Z. Part of the cell membrane is seen as the outer view, Co. In the extracellular region are numerous collagen fibrils, Cg, and a myelinated nerve axon, N, fractured to reveal the whorled membrane surrounding the axonal cytoplasm.

(ii) *Cell membranes*

Details of the muscle cell membrane are seen in figure 2. This is an inner view of the membrane showing the linear arrays of transverse tubule apertures, T, here seen as short stumps standing up from the membrane surface and casting long pale shadows. Numerous smaller rounded structures are seen covering much of the membrane. These are the sarcolemmal vesicles, V, most of which are intact and cast shorter pale shadows. Regions of the membrane not obscured by the vesicles are seen to carry numerous very small particles, P, 9 nm in diameter. Such particles are seen in replicas of many membrane types but vary in their distribution, a point referred to later.

(iii) *Internal cellular details*

Transverse tubules, T, with the associated pairs of sarcotubules, SR, forming the triads are generally radially arranged in horizontal tiers within the cells. A tangential longitudinal fracture reveals these tubules fractured transversely. A radial longitudinal fracture demonstrates these tubules either longitudinally fractured or in intact face view and not uncommonly both such views are seen in the same replica, figure 3. Here can be seen parts of three sarcomeres (muscle repeat units). The tubules of the triads lie horizontally at the Z levels at right angles to the myofilaments, F, and between successive triads run the finer longitudinal elements of the sarcoplasmic reticulum. Also in this region can be seen vertical rows of polygonal structures, the glycogen particles, G.

From a closer examination of the T tubules and associated SR tubules, it is clear that both reveal two different topographical appearances, one a concave or inner membrane surface and the other a convex or outer membrane surface, figure 3. However, whereas the outer surfaces of both tubules, To, SRo, appear relatively smooth with only a few 9 nm particles, the inner

Description of plate 26

Large arrow indicates direction of metal shadowing.

Key

Ci	inner view of the cell membrane;	P	membrane particles;
Co	outer view of the cell membrane;	SRi	inner view of sarcoplasmic reticulum;
Cg	collagen fibrils;	SRo	outer view of sarcoplasmic reticulum;
E	extracellular space;	T	transverse tubule;
F	myofilaments;	Ti	inner view of transverse tubule;
G	glycogen particles;	To	outer view of transverse tubule;
M	M-line;	V	vesicle;
N	nerve;	Z	Z-line.

Figure 1. General longitudinal fracture of part of a muscle cell showing myofilaments, F, and neighbouring extracellular material including collagen Cg and a nerve axon N.

Figure 2. Inner surface of cell membrane, Ci, revealing stumps of transverse tubules, T, numerous vesicles, V and membrane particles, P.

Figure 3. Radial longitudinal fracture, showing details of triads and sarcomeres. Note the texture of the various membrane surfaces.

Figure 4. Oblique fracture of myofibrils in the region of an M-line. The thick filaments are prominent on one side only of the M-lines.

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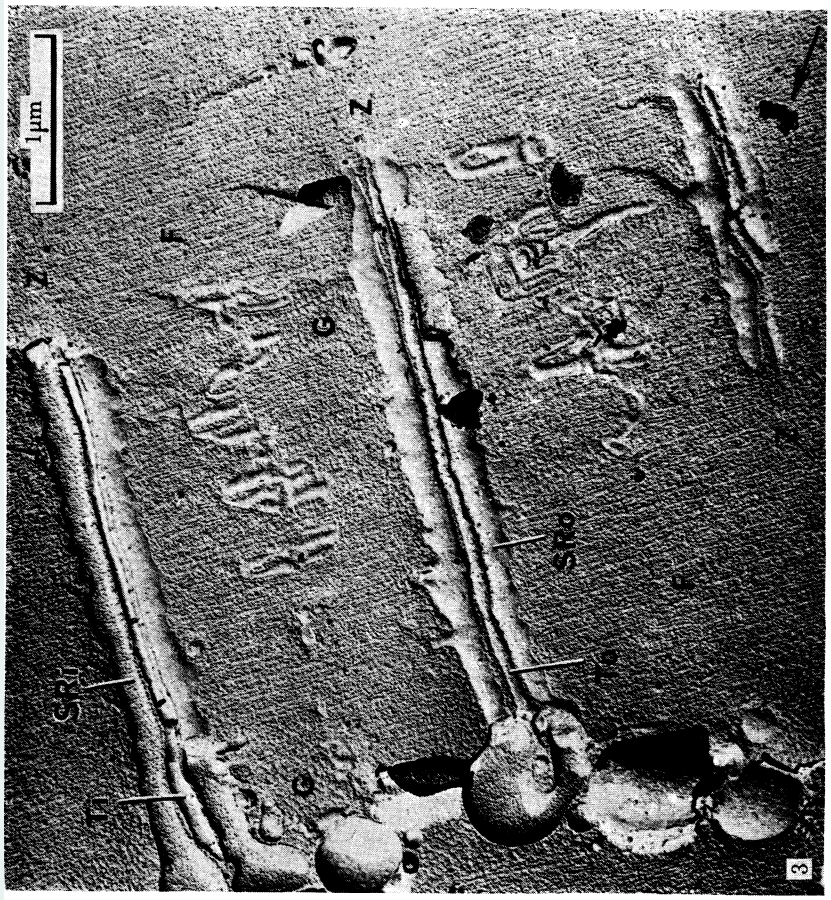

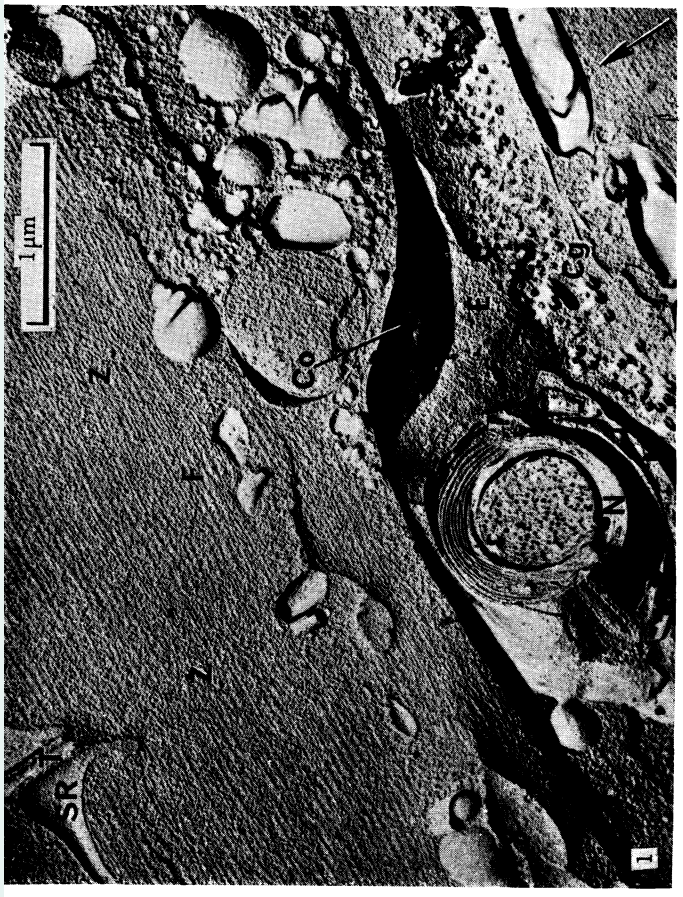

FIGURE 1. For legend see facing page.

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surfaces are strikingly different. The inner membrane of the T tubule, Ti, is again smooth and more or less particle free, but the inner surface of the SR tubule, SRi, is completely covered with closely packed 9 nm particles. Such an obvious difference in structure is perhaps to be expected in these two components of the excitation–contraction coupling system.

The longitudinal appearance of the myofilaments has been seen earlier, figures 1 and 3. However, if the fracture plane passes across a myofibril obliquely to the fibre axis, intriguing replicas of the A-band result (Bertaud, Rayns & Simpson 1968). If the fracture includes areas both sides of an M-line, there is a striking variation in the appearance of the myosin filaments. On the one side of the M-line filaments are clear and bold and raised above the general replica surface (similar to the true transverse fracture appearance). On the opposite side of the M-line, individual filaments are discerned only with some difficulty (figure 4). This phenomenon is commonly found in mammalian heart and skeletal muscles, fish body muscle and insect flight muscle. The explanation offered is as follows. The myosin filament is polarized about its mid-point, M, with component myosin molecules so arranged that the tails point towards the M region and the globular heads away from it. In one sarcomere, an oblique fracture passing across the fibril would on one side of the M line tend to fracture or chip away material by pulling at the tail ends of the molecules and on the opposite side by pulling at the head ends. The former situation tends to leave the tails behind 'anchored' by the heads in the surface to be replicated and thus produce prominent filament images, the latter situation results in the removal of tail material attached to the heads and produces an area of inconspicuous filament images. Such an explanation lends further support to the current ideas on the assembly of the myosin filament (Huxley 1963).

Discussion and conclusions

This brief survey is an attempt to show that the technique of freeze-etching besides offering pleasing three-dimensional views of striated muscle does offer new and significant information. Relatively extensive areas of curved or undulating surfaces can be visualized. This immediately allows for the quantitative analysis of small membrane surface features. For example the number of transverse tubule apertures in various muscle cell types is a matter of considerable interest to electrophysiologists; the population density of vesicles on the membranes of various muscle types (smooth and striated) and such cells as endothelium are particularly important in calculations of total membrane area. High resolution replicas of any membrane surface are most instructive with respect to the density distribution of membrane particles. This varies considerably and is currently of particular interest, especially in the study of specialized cell-to-cell junctions and differentiating membranes. Structural differences detected by this method are more obvious than the subtle differences observed by thin sectioning. Where relatively large polarized molecules are involved, these can react differentially to the fracturing process depending on their orientation with respect to the fracture plane.

It is obvious that this process of freezing, fracturing and etching particularly when employed in conjunction with other techniques will continue to be of increasing advantage in the study of biological materials at all levels of resolution.

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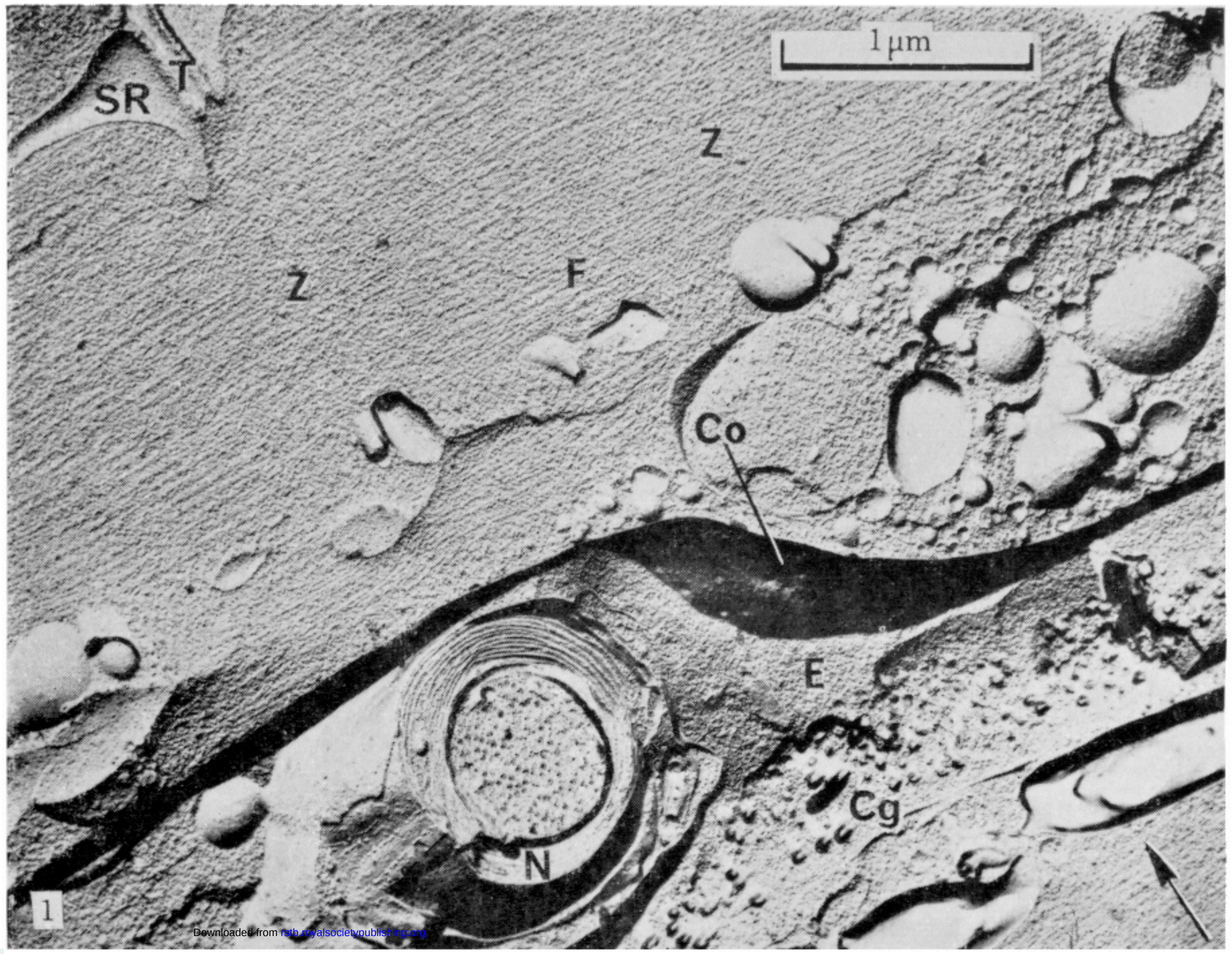

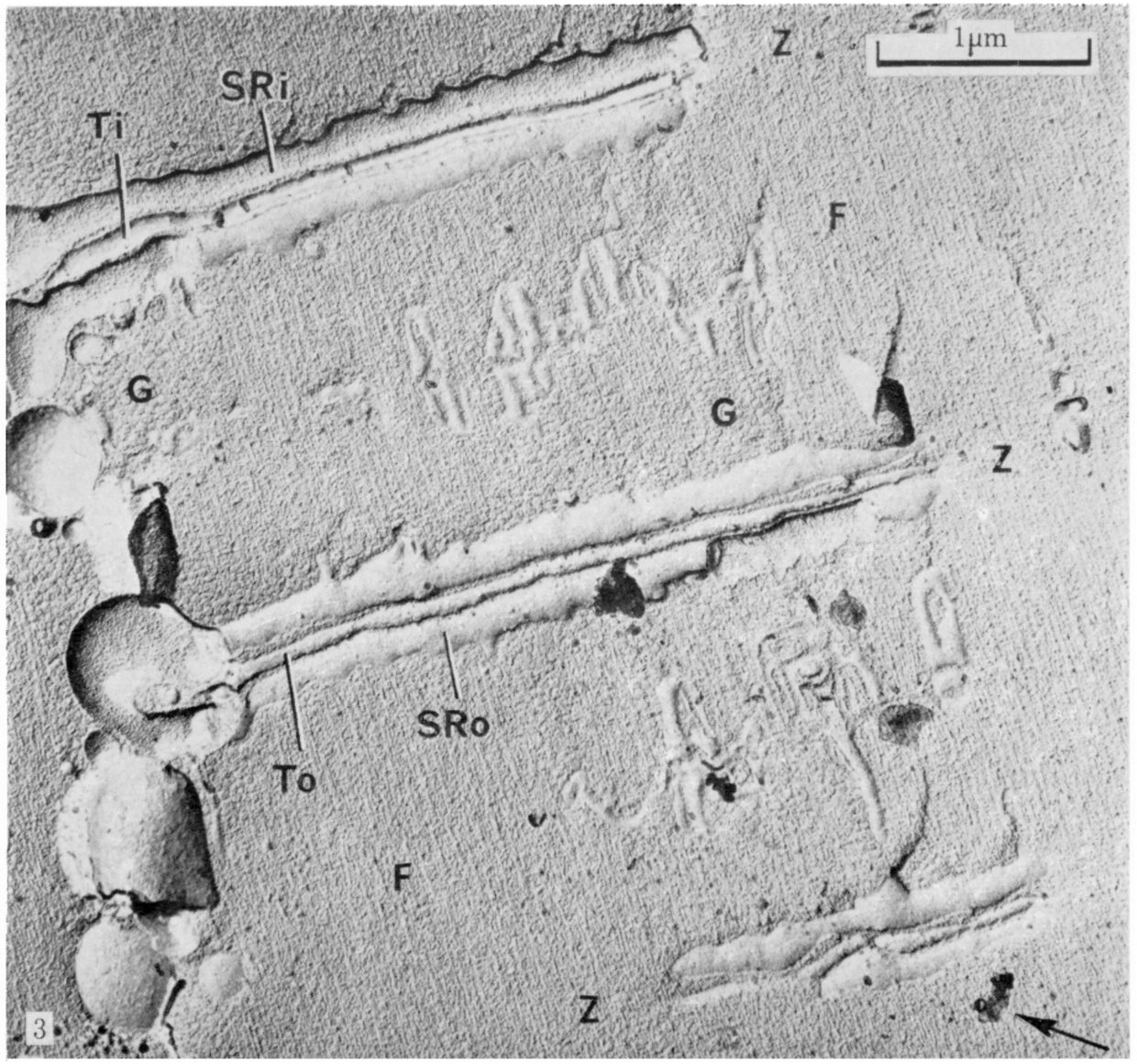

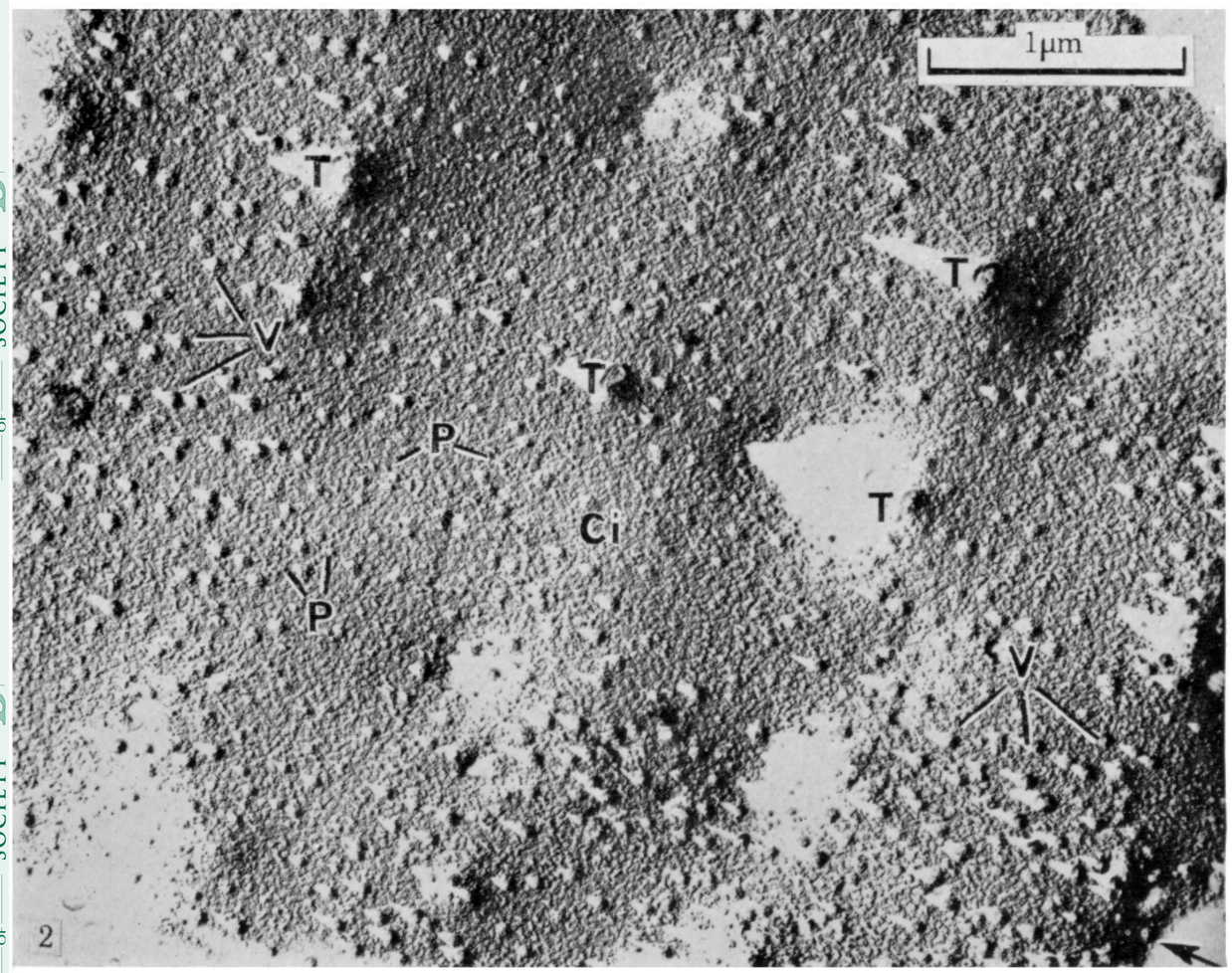

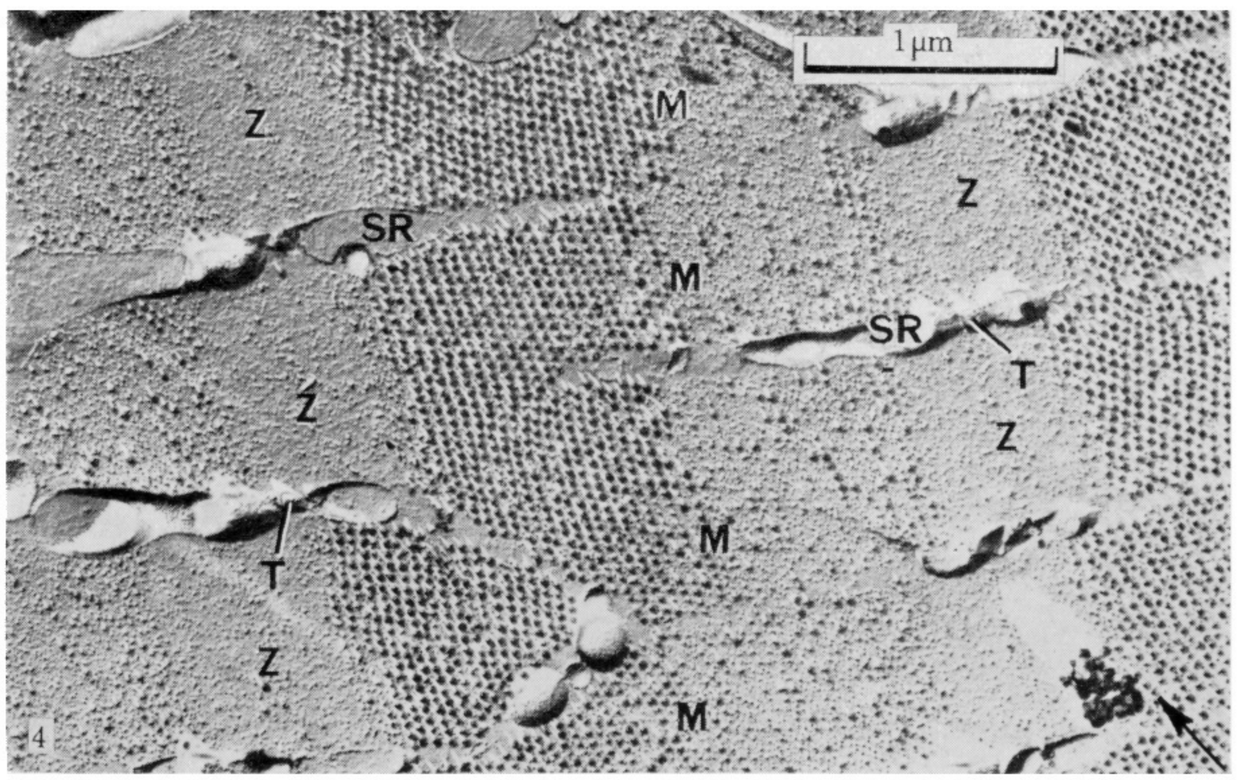

FIGURE 1. For legend see facing page.