ULTRASTRUCTURE OF DESMOSOMES IN MAMMALIAN INTERCALATED DISC; APPEARANCES AFTER LANTHANUM TREATMENT

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INTRODUCTION

At points of desmosomal contact between cells a suggestion of intercellular cross-connections has been reported (1) and an electron-opaque line is often seen midway between the apposed membranes (2, 3), but the nature of this and other extracellular components of the desmosome is still uncertain. The use of lanthanum as an extracellular marker (4) has provided new information about the structure of the desmosome.

MATERIAL AND METHODS

The present observations were made on myocardium from young male guinea pigs, anaesthetised with intraperitoneal pentobarbital. The tissue had been prepared by the following methods.

Conventional Treatment (no Lanthanum)

The heart was excised, opened, pinned out, and drip-fixed in modified Tyrode's solution (MTS; NaCl 5 g/l), containing 2% glutaraldehyde and 2% formaldehyde (1360 mOsmols, pH 7.5). After 10 min, the papillary muscles were excised, dissected, and fixed in the same fluid for 2½ hr. The specimens were washed in MTS (204 mOsmols, pH 7.3) for 2 hr, postfixed in 2% osmium tetroxide in half-strength MTS (155 mOsmols, pH 7.3) for 3 hr, dehydrated in ethanol, and embedded in Epon.

Lanthanum Treatment

The myocardium was fixed by retrograde perfusion for 3 min with 1% glutaraldehyde in MTS (335 mOsmols, pH 7.3). Pieces of papillary muscles were excised and fixed in the same fluid for 3 hr. The specimens were washed in MTS (204 mOsmols) for 2 hr, then postfixed for 2½ hr in collidine buffer (5, 4) containing 1% osmium tetroxide and 1% lanthanum nitrate (203 mOsmols). After ethanol dehydration, the specimens were embedded in Epon. Sections, estimated at 700 A in thickness (6), were examined in an Hitachi HU11A electron microscope (Hitachi Ltd., Tokyo, Japan) fitted with an HK-1S stereo stage.

RESULTS

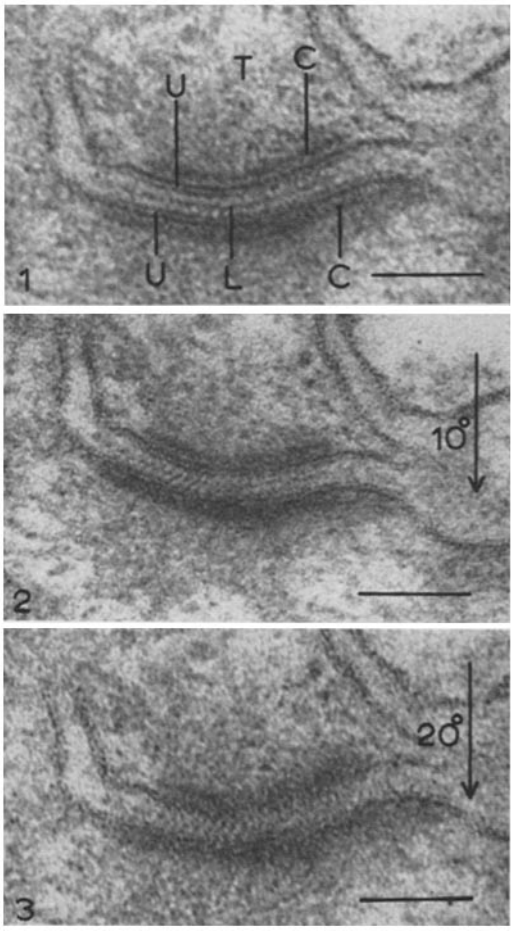
Conventionally-Prepared Material (no Lanthanum)

Perpendicular sections through desmosomes showed two trilamellar membranes running parallel to each other for distances up to 4,000 A. The gap between the centers of the two outer lamellae was about 210 A. In the cytoplasm adjacent to these regions, plaques of high density lay parallel to the cell membranes but separated from them by a less dense zone about 80 A wide (Fig. 1). Between the two cell membranes there was a central extracellular lamella of variable density and width running the full length of the desmosome, equidistant from the constituent membranes and separated from them by a less dense zone (Fig. 1).

Some desmosomes were encountered which had dense lines of 70 A periodicity running obliquely between the plasma membranes; these desmosomes appeared to have been cut in near-perpendicular section. This was confirmed by tilting a perpendicularly-sectioned desmosome (Figs. 1–3) through a total of 20°. In other desmosomes (e.g. Fig. 4), apparently sectioned more tangentially, the plasma membranes were no longer visible, and the cytoplasmic plaques were broadened to wider zones of density with ill defined extremities. Between the plaques lay a less dense region in which a series of alternating light and dark parallel lines of 70–75 A periodicity could be seen.

Lanthanum-Treated Material

The lanthanum tracer was found within the intercalated disc more consistently than at any other site. Different types of junctional complex, including desmosomes (Figs. 5–9) and close junctions (Fig. 9), could be seen from various aspects. Perpendicular sections of desmosomes (Figs. 6–8) were readily identified by their simi-



The scale on each figure represents 0.1 μ. Figures 1–4—conventionally-prepared material (no lanthanum). Figures 5–9—lanthanum-treated material.

FIGURES 1–3 A desmosome in perpendicular section viewed from three different angles. The direction and angle of tilt are indicated by the arrows. In true perpendicular section, Fig. 1, the desmosome reveals dense cytoplasmic plaques, *C*, with associated tonofilaments, *T*; the trilamellar unit membranes, *U*; and the central lamella, *L*, which longitudinally bisects the extracellular space. With progressive tilt away from this aspect, the extracellular space becomes traversed by parallel lines of 70–75 A periodicity. Aldehyde/OsO_4/lead. × 150,000.

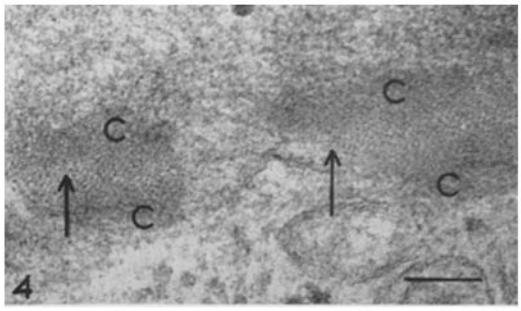


FIGURE 4 Two desmosomes sectioned obliquely. The cytoplasmic plaques, *C*, appear much broader and less dense than in Fig. 1. Parallel lines of 75 A periodicity can be discerned within the extracellular component. The dense lines are 30 A wide and the alternating pale spaces 45 A wide. There is a suggestion that the lines are composed of a series of dots in quadratic array (see arrow). Aldehyde/OsO_4/lead. × 100,000.

larity to perpendicular sections of nonlanthanum-treated material. Of the trilamellar unit membrane, only the inner and central leaflets were clearly discernible; the outer leaflets were similar in density to the extracellular lanthanum and therefore not distinguishable. Within the extracellular space, lanthanum was confined to a series of more or less spherical, extremely electron-opaque particles, 40 A in diameter, arranged in two rows, one row upon each component membrane. Particles within each individual row were separated by 30 A pale spaces. In those instances where both rows of particles were clearly visible, the particles were arranged in staggered array (Fig. 7). The central extracellular lamella appeared between the double row of dense particles as an unstained linear region with numerous short "side arms" between the particles (Figs. 7, 8). This central extracellular lamella, although appearing in negative contrast against the dense particles, was more dense than the central leaflet of the unit membrane (Fig. 7).

Tangential sections through desmosomes were identified by the presence of arrays of dense parallel lines of 75 A periodicity (Figs. 6, 9) or quadratic arrays of dense particles of 55 A center-to-center spacing (Fig. 5), associated with adjacent cytoplasmic density and frequently with tonofilaments. The desmosomes were readily distinguishable from close junctions, which displayed a hexagonal array in tangential section and a single central dense line of lanthanum in perpendicular section (J, Fig. 9).

DISCUSSION

Images of perpendicular sections of desmosomes from the two methods of preparation are readily correlated. The central extracellular lamella of conventional material is identified with the pale intermediate line in lanthanum-treated tissue, but in the latter this line can sometimes be seen to have

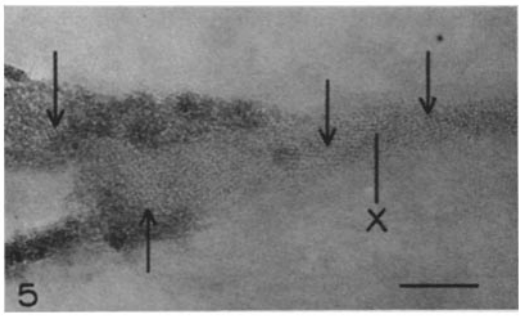


FIGURE 5 A lanthanum-treated desmosome in true tangential section. The majority of the lanthanum-stained area is seen as a quadratic array of discrete dense "particles" 40 A in diameter, clearly discernible at the arrows. *X* indicates a change in direction of the array. Aldehyde/OsO_4-lanthanum. × 100,000.

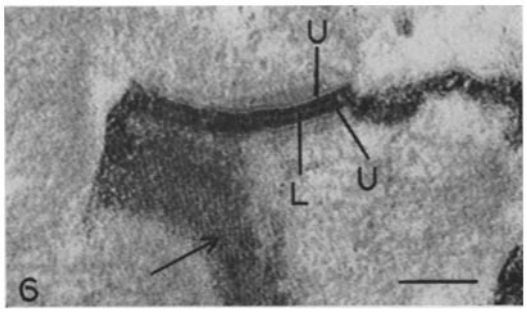
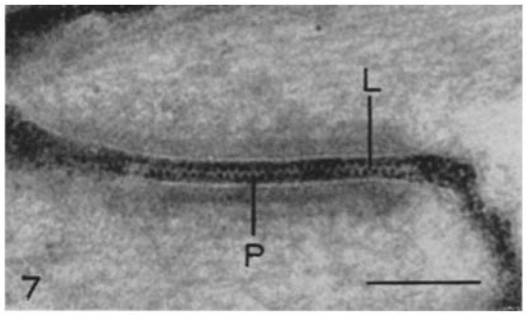
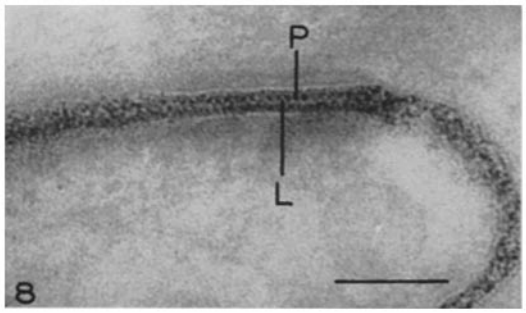


FIGURE 6 A desmosome in true perpendicular section. Within the extracellular space and adjacent to each unit membrane, *U*, are more or less continuous zones of dense lanthanum deposits. The extracellular central lamella, *L*, appears in negative contrast. Of the unit membrane, the inner leaflet is faintly stained, the middle element is pale and relatively conspicuous, but the outer leaflet is not readily distinguishable. Below this desmosome is an area of alternating dense and pale parallel lines of 70–75 A periodicity interpreted as a near tangential section of a desmosome. At some points (arrows) the dense lines are clearly composed of discrete dense particles. Aldehyde/OsO_4-lanthanum × 100,000.

side arms extending to the membranes of the two adjacent cells which are thus presumably held at a constant distance apart. These "arms" are not clearly seen with conventional staining but it is of interest that a study of ruthenium-treated desmosomes in newt epidermis (7) has revealed dense material (probably mucopolysaccharide) not only coating each cell membrane and forming a (discontinuous) central lamella but also forming cross bridges at right angles to the membranes.





FIGURES 7 and 8 Two examples of true perpendicular sections of desmosomes. The extracellular lanthanum deposits are clearly seen as arrays of discrete dense particles 40 A in diameter situated on the outer face of both cell membranes. The particles, P, in one row are staggered with respect to the particles in the companion row. The pale extracellular central lamella, *L*, comprises not only the longitudinal central element but also "side arms" extending between the particles. Aldehyde/OsO_4-lanthanum/uranium × 150,000.

These cross bridges, which may be a general feature of desmosomes (8), appear to correspond to the pale side arms of the central lamella seen after lanthanum treatment.

Images of oblique sections of desmosomes from both conventional and lanthanum-treated tissues are likewise compatible. In both instances the parallel arrays of alternating dark and light lines have periodicities of 70–80 A. In conventional material the pale lines are the broader, while after lanthanum treatment the dark lines are the broader, suggesting that the lanthanum has permeated the pale areas of conventional material.

In more truly tangential sections the lines become resolved into a quadratic array of dots which presumably in conventionally-treated tissue represent the side arms of the central extracellular lamella and in lanthanum-treated tissue the material lying as particles between the side arms.

It seems likely, as these particles become so

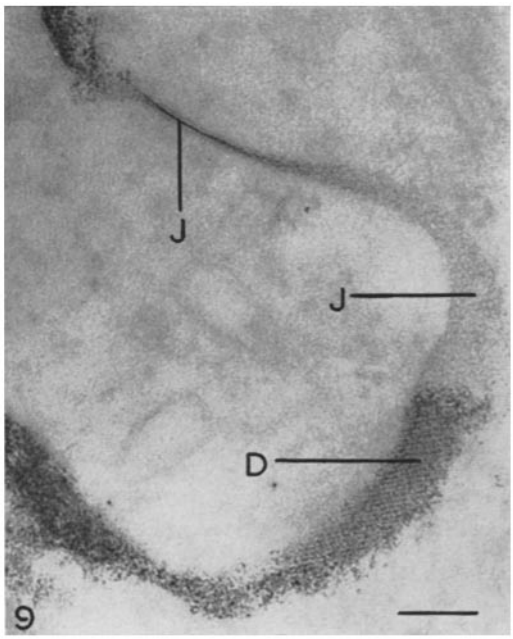


FIGURE 9 A desmosome, *D*, cut tangentially, and a contiguous close junction, *J*, cut both tangentially and perpendicularly. The desmosome is seen as a series of alternating dark and light parallel lines of 75 A periodicity. Immediately adjacent to the desmosome is an area of cytoplasmic density, but this is absent from the close-junction region. Aldehyde/OsO_4-lanthanum × 100,000.

electron-opaque after lanthanum treatment, and as lanthanum probably does not actually bind to any extracellular material (4), that the particles are freely permeable to lanthanum rather than simply coated by it. The outer leaflet of the unit membrane appears also to be permeable to lanthanum (4) and may thus provide a pathway for the lanthanum to penetrate into the apparently discontinuous spheres.

The lanthanum-stained particles are seen most clearly in either favorable perpendicular or true tangential sections of desmosomes. The appearances in perpendicularly-sectioned desmosomes are presumably governed by the relationship between the plane of section and the orientation of the side arms and particles. These structures are probably only seen when they lie in rows perpendicular to the plane of section. In such sections (e.g. Fig. 7) the particles in each row have a center-to-center spacing of 70–80 A, and this presumably represents the quadratic array spacing in each set of particles.

In tangential sections of desmosomes, with a section thickness of about 700 A and a membrane-to-membrane gap of 200 A, the entire extracellular component will usually be included. The two arrays of particles will therefore usually appear superimposed. As they are staggered with respect to each other, particles of one 80 A array will appear in between those of the second 80 A array; this will result in a center-to-center distance of about 55 A in the double array, with a 45° shift in the orientation of the array.

It is not clear whether these particles should be considered as structures in their own right or merely as spaces between the material which forms the central extracellular lamina and its side arms.

SUMMARY

In desmosomes of the intercalated disc of guinea pig myocardium, an ordered structure of the extracellular material has been demonstrated by examination of sections at various degrees of tilt and by the use of lanthanum. The central extracellular lamella is connected to the adjoining cell membrane on either side by a quadratic array of side arms; the two arrays are staggered with respect to each other. The material between the side arms appears after lanthanum treatment as electron-opaque particles, lying in similar staggered quadratic arrays.

The authors wish to thank Miss Alison Wildey for technical assistance and Dr. P. Grant for the collidine purification. This study was supported by the Medical Research Council of New Zealand.

Received for publication 20 November 1968, and in revised form 1 March 1969.

Addendum

Since this paper was prepared for publication, our attention has been drawn to the work of Dr. McAlear (McAlear, J. H., 1962. The question of the organization of substances on cellular membranes. *Ann. Histochim.* [Suppl. 2] 261), who was the first to report a regular periodic cross-structure in the extracellular component of desmosomes. Dr. McAlear's findings are probably identical with the features reported in the present paper, although his interpretation is rather different.

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