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Further observations on the cellular structure of cardiac muscle

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INTRODUCTION

A pair of cell membranes transecting the adult cardiac muscle fibre at an intercalated disc, with the myofibrils of adjacent cells inserted into these membranes, was first clearly demonstrated by Sjostrand & Anderson (1954). Confirmation of this observation in many species (Moore & Ruska, 1957; Fawcett & Selby, 1958), in conducting tissue fibres (Muir, 1957*a*; Rhodin, del Missier & Reid, 1961; Hayashi, 1962) and in embryonic myocardium (Muir, 1957*b*) leads to the conclusion that cardiac muscle is composed of discrete cells. A review of this subject (Sjostrand & Andersson-Cedergren, 1960) shows that there is no valid evidence in favour of the 'syncytial' concept, that is complete cytoplasmic continuity along the fibres, although this view was widely accepted after the incorrect interpretation of the information available from the light microscope (Heidenhain, 1901; Jordan & Banks, 1917).

It should be recognized, however, that electron microscopy cannot exclude quite extensive continuity from cell to cell. Complete serial sections of an intercalated disc are not available, so a localized zone of continuous cytoplasm could be present even though most of the fibre is transected by paired membranes. Technical factors can produce imperfections in the disc membranes and even in the best preparations the tortuous course of these membranes means that, when they are obliquely sectioned, the cytoplasm may appear to be continuous through the disc. These possibilities have been considered by observing the permeability of the intercellular barriers to the high glycogen content of cardiac muscle, Yokoyama, Jennings & Wartman (1961) showing that the glycogen-flight due to fixation is arrested at the intercalated discs in dog myocardium. In the present paper a centrifugation technique is used to study the movement of cytoplasmic material in sheep Purkinje fibres.

If cardiac muscle is composed of discrete cells, the implications of this structure on the physiological properties of the myocardium must be considered. In particular, the mode of propagation of the impulse for contraction along normal and conducting tissue fibres should be interpreted in relation to the presence of intercellular partitions at the intercalated discs. In a discussion of this problem, Woodbury (1962) does not consider the different types of intercellular relationship observed within the discs (Sjostrand, Andersson-Cedergren & Dewey, 1958; Karrer, 1960). Improved methods of fixation and embedding are used in the present study of these intercellular contacts and the results are interpreted according to the current concepts of cell membrane structure (Robertson, 1960; Sjostrand, 1960).

Further information on the nature of these cellular junctions is provided by the study of the fine structure of the ends of cardiac muscle cells which have been

dissociated by the method of calcium ion chelation (Yokoyama *et al.* 1961). This technique also provides information on the size and shape of individual cardiac muscle cells.

MATERIAL AND METHODS

Normal myocardium from the ventricles of adult rats, sheep and hens and Purkinje fibres from the ventricles of the sheep heart have been used in these experiments.

Centrifugation experiments

Immediately after slaughter, the septomarginal trabeculae were dissected from the right ventricles of sheep hearts. The trabeculae were stored at 4° C. for up to 1 hr. before being attached to wooden supports for centrifugation. The centrifuge had a vertical-sided funnel fixed to the spindle, with outlets flowing into the buckets so that fixation could be effected during centrifugation. The specimens were centrifuged at 4500 rev./min., giving a mean centrifugal force of 2900 **g**, for 20 min. before fixation with Susa and centrifugation at 2000 rev./min. was continued for the first $\frac{1}{2}$ hr. of fixation. Control specimens were similarly fixed to wood in centrifuge tubes and the fixative added after 20 min. After fixation, the centrifugal pole was marked and the specimens embedded in paraffin wax. Glycogen was demonstrated by the periodic acid-Schiff reaction (Pearse, 1960) the sections being counterstained with haematoxylin.

Electron microscopy

Slender false tendons from the sheep's ventricles were immersed in buffered 1 % osmium tetroxide for 1 hr. Rat's hearts were also fixed by immersion but more satisfactory results were obtained by perfusing the fixative. The aorta was cannulated and the coronary circulation washed out with 0·9 % saline at 37° C. for 2 min. The beating heart was then perfused for 10 min. at 37° C. with a balanced salt solution aerated with 95 % O_2 and 5 % CO_2. The composition of the balanced salt solution was 0·69 g. NaCl, 0·2 g. $NaHCO_3$, 0·028 g. $CaCl_2,6H_2O$, 0·016 g. KH_2PO_4, 0·015 g. $MgSO_4.7H_2O$ and 0·035 g. KCl per 100 ml. distilled water. Fixatives, at room temperature, were injected through the cannulae and the intact heart left in the fixative for $\frac{1}{2}$ hr. A variety of fixatives were employed, 1% osmium tetroxide, 10 % formalin, 10, 5 and 2 % glutaraldehyde in veronal-acetate (Palade 1952), veronal-acetate-sucrose (Caulfield, 1957), chromate-dichromate (Dalton, 1955) and phosphate buffers (Sabatini, Bensch & Barrnett, 1963). The results reported were observed in all the specimens but perfusion with 2% glutaraldehyde in the balanced salt solution described above gave the most satisfactory results. After fixation, blocks less than 0·5 mm. thick were cut from the ventricle wall, and the glutaraldehyde and formalin fixed specimens were post-osmicated for 1 hr. in 1% osmium tetroxide.

Ethanol dehydration was followed by Araldite embedding and sectioning on a Porter–Blum microtome equipped with a glass knife. One micron thick sections, mounted on slides, were stained with toluidine blue-pyronin (Ito & Winchester, 1963) for light microscopy. Electron microscope sections were mounted on Athene

483 grids, without a supporting membrane, and stained with either potassium permanganate (Lawn, 1960) or uranyl acetate-lead citrate (Reynolds, 1963) before examination in an A.E.I. EM 6 microscope.

Dissociation of myocardial cells

The technique described by Yokoyama *et al.* (1961) was employed on rat, sheep and fowl ventricular muscle. The fresh tissue was minced with scissors, until the pieces were about 1 $mm.^3$ in size and they were gently agitated for 1 hr. in a solution containing 0·0027M-ethylene diamine tetra-acetate (EDTA), 0·25M-sucrose and 0·2M-tris (hydroxymethyl) aminomethane-maleate buffer at a pH of 7·4. At the end of this period the tissue was ground up for 5 sec. in a Waring blender operated at full speed. Undissociated fragments were allowed to settle, then the supernatant was gently centrifuged to bring down the isolated cells. The deposit was resuspended in glycerol for examination in the phase contrast microscope. For electron microscopic examination, the deposit was resuspended in 1% osmium tetroxide in the veronal-acetate-sucrose buffer (Caulfield, 1957) for ½ hr. The osmium fixed cells were then centrifuged into a pellet, the fixative discarded and a few drops of warm fluid bacteriological agar added to the centrifuge tube. When the agar had cooled and set, the agar block was cut up for embedding in Araldite. Smears of the dissociated cells were fixed in 10% formalin for staining with the Feulgen reaction or haematoxylin.

RESULTS

Centrifugal movement of 'glycogen' in Purkinje fibres

A centrifugal force of 2900 **g** applied for 20 min. to the strands of Purkinje fibres contained in the septomarginal trabecula of the right ventricle of the sheep's heart does not aggregate the PAS-positive material at the centrifugal pole of the trabecula. The centrifugal movement appears to be arrested by transverse partitions, which correspond to the intercellular junctions, as judged by the distribution of nuclei along the Purkinje fibres (Pl. 1, fig. 1). A partial arrest of this movement by incomplete partitions would be expected to produce a similar histological pattern. However, examination of the control specimens shows that in the uncentrifuged strands, there is considerable variation in the concentration of stainable glycogen on the two sides of the intercellular junctions (Pl. 1, fig. 2). In conjunction, these observations suggest that the intercellular junctions are impermeable to stainable glycogen particles.

These studies provide a method of revealing the length of Purkinje cells. On the centrifuged strands, measuring the distance from one abrupt change in concentration to the next gives a mean length for 57 individual Purkinje cells of 165 μ (99–240 μ). The distinction between adjacent cells is less certain in the control specimens where mean value of 132 μ (72–168 μ) is obtained.

Intercellular relationships in normal myocardium

The sides of a myocardial cell are related to the extensive extracellular space which separates the parallel muscle fibres. Each of these fibres is formed by the end to end junction of a series of single cells; at the junctions the cells are closely applied to each other to form intercalated discs.

The surface membrane on the side of the osmium-impregnated cells is seen to be composed of an osmiophilic lamina (Pl. 2, fig. 4, *I.P.M.*), which is less than 6 mμ thick and has a clear interval of about 15 mμ separating it from an outer amorphous layer which is less dense and has no sharp boundaries. The trilaminate structure of the plasma membrane, seen after permanganate fixation (Robertson, 1960), could not be observed after osmium impregnation and, for reasons mentioned later, it is considered that the osmiophilic lamina represents the inner dense layer of the three components of the plasma membrane. The remaining layers of the plasma membrane occupying part of the clear gap outside this osmiophilic layer, such assymmetry in the appearance of the plasma membrane has recently been demonstrated by Sjostrand (1963). No satisfactory term exists for the amorphous outermost layer; in structure it resembles epithelial basement membranes and it will be thus designated (Pl. 2, fig. 4, *B.M.*). The sarcolemma consists then of a plasma membrane, an intervening gap and the basement membrane while the endomysium is represented by the scattered collagen fibres in the intercellular space. It is noted that pinocytotic vesicles (Pl. 2, fig. 4, *V.*) on the surface of the cell only affect the plasma membrane and that myofibrils and other cytoplasmic constituents abut directly on the inner surface of the plasma membrane.

At the ends of the cells, where an intercalated disc is formed, the basement membrane passes without interruption or invagination on to the surface of the next cell (Pl. 2, fig. 5, *B.M.*). The plasma membranes, however, turn inwards to traverse the fibre with a clearer space between the opposed membranes. A low magnification view of a longitudinally sectioned intercalated disc is shown in Pl. 1, fig. 3, the disc always crosses the myofibrils where a Z-band would be expected and it frequently crosses adjacent myofibrils at different Z-band levels, so giving the whole disc a step-like course across the fibre. Even at this magnification, the myofibrils on either side of the disc can be seen to end in aggregations of electron-dense material which are applied to the inner surfaces of the opposed membranes. The tortuous course of the membranes with the dense material applied to them creates a structure which can be readily seen by light microscopy. Where the membranes run parallel to the myofibrils, they are less obvious in such an electron micrograph and are invisible by light microscopy, thus giving the disc an artificial appearance of discontinuity.

Closer examination of the opposed membranes of adjacent cells within the disc shows that four distinct types of interrelationship are present (Pl. 3, fig. 7). There is the normal intercellular relationship (Pl. 3, fig. 7, *G.*), with the plasma membranes following an irregular course separated from each other by a gap which is not less than 20 mμ wide and which, after fixation with perfused glutaraldehyde, often shows local dilatations (Pl. 1, fig. 3, *G.*). There are, in addition, three specialized types of junction called the myofibrillar insertion plaques (*P.*), the desmosome (*D.*) and the quintuple-layered membrane junction (*Q.*) (Pl. 3, fig. 7). Between the myofibrillar insertion plaques, a constant space of about 20 mμ separates the membranes as they cross the myofibrillar axis (Pl. 4, fig. 8; Pl. 3, fig. 7, *P.*). The thin filaments of the *I*-bands from the last sarcomeres of each cell disappear into the dense material of the plaques, which does not show any lamination.

The desmosomes (Pl. 3, fig. 7; Pl. 4, fig. 8, *D.*) resemble the myofibrillar insertion plaques, but they do not receive myofilaments and there are differences in their fine

structure. The dense material applied to the membranes in a desmosome is laminated and in the gap between the membranes three thin dark laminae can be distinguished. Cytoplasmic filaments, not belonging to the myofibrils are often seen to be attached to the desmosomes.

At the quintuple-layered membrane junctions (Pl. 3, fig. 7; Pl. 4, figs. 9, 10 and 11, *Q.*) the two osmiophilic layers of the plasma membrane approach each other and run parallel for a distance of up to 1·5 μ. The overall thickness of the quintuple unit is about 15 mμ and the centre to centre distance of the limiting dark layers is 12 mμ. The central element of the five layers can appear as a thin line (Pl. 4, fig. 9, *L.*) or a series of dots (Pl. 4, fig. 11) the spacing of the dots being about 9 mμ. Permanganate staining of the sections shows the central element more clearly than either lead citrate or uranyl acetate, and its appearance is the same in sections cut parallel and transverse to the myofibrils. The above structure is only observed when the membranes are parallel to the electron beam, slight inclination producing the image shown in Pl. 4, fig. 10, where a central density is produced by the overlapping of the lower edge of one membrane by the upper edge of the other membrane. Further inclination causes a disappearance of internal structure as in the upper part of Pl. 4, fig. 9, with the resolution so far attained, no surface pattern has been detected on these osmium impregnated membranes.

The dimensions and structure of the quintuple-layered membrane junctions suggest that they are formed where the outer layers of two triple-layered plasma membranes come into close contact. This interpretation, which is supported by observations between epithelial cells (Farquhar & Palade, 1963; Muir & Peters, 1962) and in the myelin sheath (Peters, 1962), does imply that the osmiophilic layer covering the rest of the cell is merely the inner layer of the whole plasma membrane. It should be noted that, at the quintuple-layered junction, both the inner and outer layers of the plasma membrane are modified, the two outer layers becoming obvious as a line or series of dots and the two inner layers having a greater density and thickness than over the rest of the cell.

The quintuple-layered junctions are usually seen between the paired membranes running parallel to the myofibrils, hence they are more numerous when the disc shows many steps in its course across the fibre. As serial sections through a whole fibre have not been obtained, it is difficult to determine whether the quintuple-layered junctions form continuous bands surrounding islands of unspecialized membranes or are only localized zones of contact. The latter seems most likely, since a longitudinal section of a disc (Pl. 1, fig. 3) can show six, separate, randomly distributed junctions and a complete circle of membrane fusion is not seen in transverse sections. Although the junctions are seen at the edge of a disc, they are often absent as in Pl. 2, fig. 5, hence sections taken near the periphery of a disc may not show any such contacts. Sections of the myocardium of a 14-day rat embryo show quintuple-layered junctions with the same structure and dimensions as in the adult.

The parallel fibres of normal myocardium are usually separated from each other by considerable intercellular spaces containing capillaries, collagen and fibroblasts. Occasionally two parallel fibres approach each other and, although there is no myofibrillar attachment, a sarcoplasmic projection makes contact between the two

fibres (Pl. 2, fig. 6). The basement membranes of the two fibres are continuous with each other, but the plasma membranes form quintuple-layered junctions and desmosomes between the adjacent sides of the cells. The desmosomes are usually seen at the level of the *Z*-bands of the myofibrils.

Intercellular relationships of Purkinje fibres

A bundle of Purkinje fibres contains several strands of cells surrounded by a collagenous sheath, with little extracellular space and no capillaries intervening between the adjacent strands of the bundle. Thus, only the outer surface of the outermost strands is covered with basement membrane similar to that covering the sides of a normal myocardial cell. Within the bundle all the surfaces of the Purkinje cells are related to each other by structures which are only found at intercalated discs in normal myocardium. Myofibrillar insertion plaques (Pl. 5, fig. 12, *P.*) are seen only where the peripheral myofibrils are attached, but desmosomes are more numerous than in normal myocardium and are always associated with fine fibrils which form a delicate meshwork throughout the Purkinje cell sarcoplasm (Pl. 5, fig. 13, *F.*). At the ends and along the sides of the Purkinje cells, quintuple-layered junctions with the same structure and dimensions as in normal fibres are found (Pl. 5, fig. 13, *Q.*). The remainder of the adjacent membranes are more closely related to each other than in the unspecialized areas of normal discs, for the clear gap between the membranes rarely exceeds 13 mμ and the centre to centre distance of the membranes is about 17 mμ (Pl. 5, fig. 13, *G.*). The area of such contacts between cells is greatly increased by interdigitation of the sides and ends of adjacent cells.

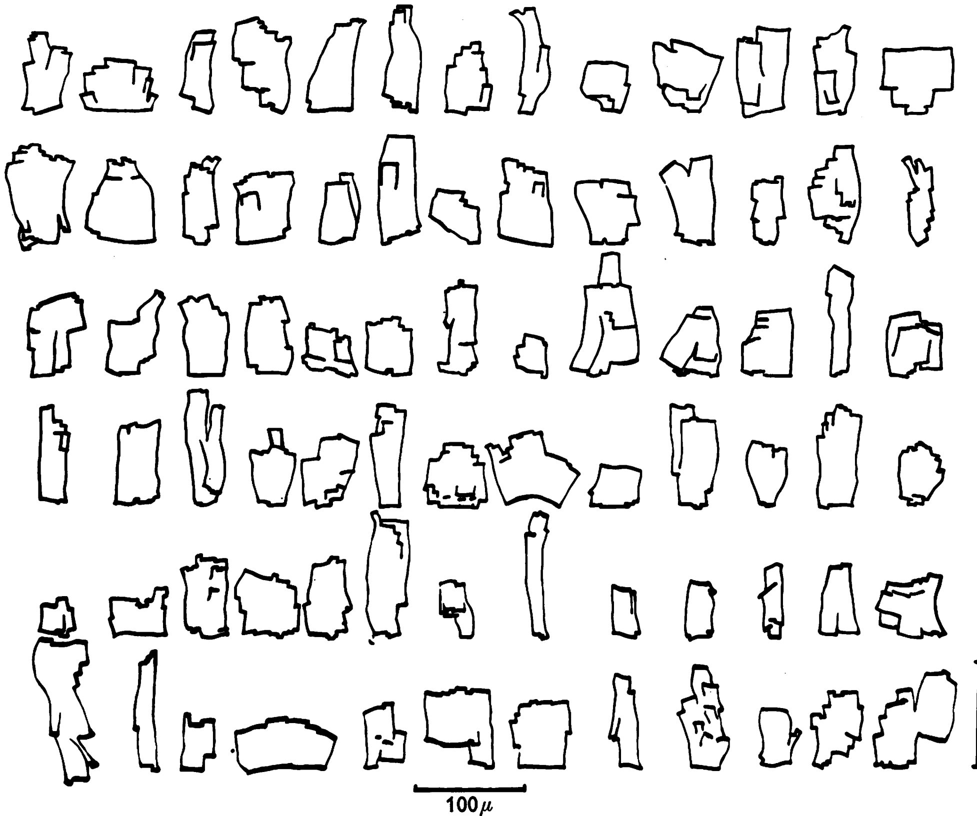
Dissociation of myocardial cells

A method of separating individual cardiac muscle cells from the ventricles of the dog heart by chelating calcium ions with EDTA and subsequent brief agitation in a Waring blender is reported by Yokoyama *et al.* (1961). These authors do not describe the shape of the isolated cells or the electron microscopic appearance of the separated intercalated discs.

When examined by phase contrast microscopy, the typical step-like ends of the isolated segments (Pl. 6, fig. 14, *I.D.*) suggest that they have dissociated at their intercalated discs. This is confirmed by examining such segments after osmium fixation, embedding and sectioning (Pl. 6, fig. 16) when the dense material of the myofibrillar insertion plaques is seen at the end of the segment. Using the experimental conditions described above, the suspension of isolated segments contains some smaller debris and occasionally two or three cells are seen to be adherent by noticing the undissociated intercalated discs in the segment. By ignoring such contamination, outline camera lucida drawings of individual cells under phase contrast can be obtained and are shown in Text-fig. 1. A stained smear of such specimens shows that each cell usually contains two nuclei (Pl. 6, fig. 15) although occasionally only one is present. Similar preparations can be obtained from adult sheep and hen ventricle, but a 140 mm. foetal sheep heart yields spindle-shaped cells with only one nucleus.

The length of the adult rat ventricular cells range from 130–35 μ, corresponding to about 80 to 20 sarcomeres along the myofibrils between the discs. This range

agrees with random counts made on low-power electron micrographs of intact rat ventricular muscle. There is apparently great variation in the width of the cells, 12–90 μ, the wider specimens are not cylindrical, their thickness being much less than their width.



Text-fig. 1. Camera lucida outlines of 77 cardiac muscle cells from the ventricles of an adult rat heart. Unfixed and mounted in glycerol for phase contrast examination.

A high-power micrograph of a dissociated disc is shown in Pl. 6, fig. 17, the preparative procedure produces some damage to the cells but a myofibril and its insertion plaque can be seen in the lower part of the field, the corresponding part of the other cell having disappeared. In the upper part of the field a quintuple-layered junction is present, its overall thickness is 14 mμ and a faint central line can be seen on the original print. The right-hand half of this junction is thus derived from the adjoining cell and it has broken off from its own cell during dissociation. Remnants of desmosomes could not be identified on the ends of these dissociated cells.

DISCUSSION

Although the evidence from electron microscopy and cellular dissociation seems to show that the myocardium is composed of separate cells joined at the intercalated discs, these methods cannot exclude the presence of some cytoplasmic continuity

between adjacent cells. The observations which suggest that the cells are discrete are that the micro-injection of saline into cardiac muscle fibres is arrested only by the intercalated discs (de Renyi, 1945) and that the degree of contraction and extent of pathological change (Yokoyama *et al.* 1961) may change abruptly on either side of a disc. The movement of stainable glycogen due to fixation artifacts is impeded by the discs in dog myocardium (Yokoyama *et al.* 1961) and it is here confirmed that similar barriers across the fibre axis are present in sheep Purkinje fibres. All these observations would seem to exclude any extensive cytoplasmic continuity and the evidence from the glycogen experiments suggests that if any minute pores are present between adjacent cells, then their diameter is appreciably less than that of particulate glycogen.

The demonstration of a discrete cellular structure must be reconciled with the clear physiological evidence of myogenic transmission of the cardiac impulse. Many observations suggest that the opposed membranes at the discs have different electrical properties to those present in the sarcolemma at the sides of the cells. Using goat and cat ventricular muscle, Draper & Mya-Tu (1959) show that the conduction velocity along the fibre axis is 0·6 m./sec. while transverse to the axis it is only 0·2 m./sec., these authors also noted that, when measuring the conduction rate transverse to the axis, closely related but different values are obtained. It is suggested that this variation is due to the impulse taking alternative pathways through the interlinked parallel muscle strands, an interpretation which would clearly support preferential transmission across the discs. Similarly, Crill (1960) shows that when a current is applied through an intracellular electrode in rat atrial muscle, the equipotential contour lines around this point are elliptical with their long axes oriented along the fibres. Wiedmann (1952) demonstrates a low electrical resistance along Purkinje fibres, but since it is technically impossible to insert micro-electrodes into two adjacent cells, direct measurement of disc resistance cannot be made. Studies on the migration of ^{42}K along strands of Purkinje fibres indicate a low intercellular resistance to the passage of this ion (Wiedmann, 1960, 1964).

Any consideration of the transmission of the impulse should be related to the types of intercellular contact which are observed at the discs. First, it is necessary to define which element of the cell surface, as seen on electron micrographs, is concerned in maintaining the ionic imbalance responsible for the action potential. The triple-layered unit membrane, observed after permanganate fixation (Robertson, 1960) is the only surface structure always found on higher animal and plant cells, its chemical composition and dimensions in the myelin sheath (Finean, 1960) conforming to the lipoprotein model for the plasma membrane proposed by Danielli & Davson (1935). Sjostrand (1963) has recently shown that the uniform structure seen after permanganate fixation is not observed in osmium preparations, the inner and outer laminae of the unit membrane may show different affinities for osmium and so confer an asymetrical structure to the membrane. In the sarcolemma of cardiac muscle the single osmiophilic lamina seen after osmium impregnation is thought to represent the inner lamina of the triple-layered unit membrane and so it is considered that this lamina outlines the intracellular ionic medium.

Within the discs, four types of intercellular contact between the plasma membranes are reported in the present study, namely desmosomes, myofibrillar insertion

plaques and quintuple-layered membrane junctions as well as the normal intercellular gap of at least 20 mμ. The nature of the material present in this gap is disputed, Sjostrand *et al.* (1958) consider it to be hydrophobic and lipid in nature, however, in the toad retina Lasansky & Wald (1962) show experimentally, using a ferrocyanide method, that similar gaps are permeated by extracellular fluid. It would seem unlikely that changes in the ionic permeability of one membrane would be transferred across an intervening aqueous space and there is no concentration of vesicles at the discs which would suggest a synapse using chemical transmitter substances.

Desmosomes are widely distributed in epithelial tissues, Farquhar & Palade (1963) suggest the term 'macula adhaerens' for these laminated, discrete areas of cellular adhesion. In normal myocardium (Sjostrand *et al.* 1958) and in conducting tissue fibres (Rhodin *et al.* 1961), it is suggested that these structures, also known as *S*-regions, are concerned with propagating the impulse. The latter authors suggest that the increased number of desmosomes in Purkinje fibres is responsible for the more rapid conduction.

Quintuple-layered membrane junctions are similarly widely distributed, occurring between many types of cell where they are known by a variety of names and thought to fulfil many functions. Between epithelial cells (Farquhar & Palade, 1963) and endothelial cells (Muir & Peters, 1962) these fusions may prevent intercellular diffusion and the name 'zonula occludens' is applied by Farquhar & Palade. Such junctions between glial cells (Gray, 1961; Peters, 1962) may fulfil a similar role in relation to the blood-brain barrier. The lymphocytes within the thyroid gland in auto-immune disease are applied to the epithelial cells by the same close relationship (Irvine & Muir, 1963), and it is suggested that the transfer of specific antibodies may take place at such regions. Karrer (1960) illustrates these quintuple-layered junctions between cardiac muscle cells in the walls of the great veins of mice and he suggests their possible role in impulse conduction. Sjostrand *et al.* (1958) had previously described the side to side contacts as 'longitudinal connecting surfaces' in mouse and guinea-pig cardiac muscle. The term, nexus, is applied by Dewey & Barr (1962) to these junctions between excitable cells; they observed them between smooth muscle cells in the dog jejunum. A purely adhesive function is ascribed to this type of junction between fibroblasts in tissue culture (Devis & James, 1964).

Direct experimental proof that these junctions limit intercellular diffusion is provided by Farquhar & Palade (1963), who show that haemoglobin and zymogen secretions will penetrate between epithelial cells only as far as the fused membranes. Otherwise the suggested functions of these contacts are based only on their form and distribution. There is, however, a close correlation between the occurrence of these fusions and the presence of electrotonic transmission in smooth muscle tissue and between neurones. Evans & Evans (1964) show that quintuple-layered junctions are present in the single unit smooth muscle of the amnion but absent in the profusely innervated, multi-unit nictitating membrane muscle. Such junctions do not occur at motor end plates or at normal, chemically mediated synapses in the central nervous system, but in a recent review of electrotonic synapses Eccles (1964) states that this form of transmission occurs on the motor neurones of the crayfish and the Mauthner cells of the goldfish (Furshpan & Potter, 1959). At these particular synapses, Robertson has demonstrated quintuple-layered membrane fusions, which

he calls external compound membranes (Robertson, 1961; Robertson, Bodenheimer & Stage, 1964; Robertson, 1964). On the Mauthner cells, after permanganate fixation, Robertson (1964) shows that the opposed fused membranes have a surface pattern, which in transverse section appears as a central beading with a repeating period of 8·5 mμ. It is here demonstrated that the central element of the quintuple-layered junction between cardiac muscle cells, after osmium fixation, appears as a beaded structure with a repeating period of 9 mμ. The present observation that, after calcium ion chelation and cellular dissociation, the component membranes of two cells remain adherent indicates a definite bond between the membranes and the thickening of the inner layer of the two plasma membranes suggests a structural alteration at these points of fusion.

The intercellular ionic continuity, which is necessary for the low electrical resistance of the discs and electrotonic transmission, could be attained by these membrane fusions in two possible ways. They could act as a 'zonula occludens' around an isolated area of ionically permeable membrane or they could contain, within the area of fusion, aqueous pores able to transmit ions between the cells. The arrangement of the fusions in the intercalated discs does not suggest any peripheral distribution and the presence of pores large enough for hydrated alkali metal ions, with a diameter of less than 0·5 mμ, extending through the paired membranes for their combined thickness of about 15 mμ could rapidly escape detection with the current procedures.

Consideration of the membrane relationships of Purkinje fibres reveals some differences of possible electrophysiological importance from those obtaining in normal myocardium. The length of sheep Purkinje cells does not differ significantly from normal myocardial cells, but with bundles of these parallel cylindrical cells bound in a connective sheath the architecture of the conducting tissue fibres is quite different from the single, branching strands of flattened cells in normal myocardium. Rhodin *et al.* (1961) and Hayashi (1962) illustrate these features clearly in their studies of the impulse conducting system of the bovine heart. The contacts between the sides of the Purkinje fibres include desmosomes and quintuple-layered junctions and the numbers of these are increased by the plication of the opposed membranes. This morphology would suggest that, if either of these modifications is concerned with ion transmission, the ohmic resistance transverse to the axis within a compact bundle should be the same as along the fibre and thus that the effective diameter of the fibre is the diameter of the whole bundle. There is little extracellular space within the bundle, large spaces are eliminated and even the normal intercellular space, where present in electron micrographs, is reduced from 20 to 13 mμ. It is of interest that Weidmann (1961) postulates a narrow extracellular space around Purkinje fibres to support his suggestion that a local increase in potassium ion concentration is responsible for the termination of the cardiac action potential.

According to its derivation, the term 'syncytium' means fused cells and is thus an accurate description of the relationship between cardiac muscle cells. However, as the term is often used to indicate extensive cytoplasmic continuity or even an absence of any cellular compartments, it is not wise to apply it to heart muscle where the fusion may only extend to a limited ionic transfer.

SUMMARY

1. The movement of stainable glycogen in Purkinje fibres is arrested by intercellular partitions, thus demonstrating that these fibres are composed of discrete cells.

2. At the intercalated discs between normal myocardial cells and between Purkinje cells, four types of intercellular relationship are observed. Dense plaques applied to the membranes receive the insertion of myofibrils, desmosomes not associated with myofilaments are present and the remaining parts of the plasma membrane either have an intercellular gap of 15–20 mμ or they fuse to form a quintuple-layered membrane junction.

3. After dissociation of cardiac muscle cells, the size and shape of the usually binucleate cells can be demostrated. The separated intercalated discs show that the quintuple-layered membrane junctions, derived from the plasma membranes of the two cells, remain adherent to only one cell.

4. The significance of these observations on the intercellular spread of the cardiac impulse is discussed and attention is drawn to the similarity between the quintuple-layered junctions and the structure of electrically mediated synapses in the central nervous system.

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EXPLANATION OF PLATES

Key to lettering

B.M.	Basement membrane	*D.*	Desmosome
F.	Sarcoplasmic filaments	*G.*	Gap between plasma membranes
I.	Thin filaments of *I*-band	*I.D.*	Intercalated disc
I.P.M.	Inner layer of the plasma membrane	*P.*	Myofibrillar insertion plaque
Q.	Quintuple-layered membrane junction	*V.*	Pinocytotic vesicle
Z.	*Z*-band		

Normal myocardium from the epicardial surface of the ventricles of adult rats and Purkinje fibres from the septomarginal trabecula and false tendons of the sheep heart are shown in these illustrations.

PLATE 1

Figs. 1 and 2. The Purkinje fibres in fig. 1 were subjected to a centrifugal force in the direction of the arrow prior to fixation, fibres in fig. 2 are from a control specimen. PAS. × 350.

Fig. 3. Longitudinal section of an intercalated disc and adjoining parts of two heart muscle cells from normal myocardium. Fixed by perfusion with 2 % glutaraldehyde, post-osmicated and section-stained with uranyl acetate. × 15,000.

PLATE 2

Fig. 4. Transverse section of a normal ventricular fibre showing the sarcolemma. Fixed in 10 % glutaraldehyde, post-osmicated and section-stained with potassium permanganate. × 62,000.

Fig. 5. The peripheral part of an intercalated disc in normal myocardium. Fixed in 10 % glutaraldehyde, post-osmicated and section-stained with lead citrate and uranyl acetate. × 55,000.

Fig. 6. Longitudinal section of two normal myocardial fibres, showing an adhesion between the lateral surfaces of the cells. Fixed in 2 % glutaraldehyde, post-osmicated and section-stained with lead citrate and uranyl acetate. × 32,000.

PLATE 3

Fig. 7. Longitudinal section of a portion of an intercalated disc from normal myocardium. Fixed by perfusion in 2 % glutaraldehyde, post-osmicated and section-stained with lead citrate and uranyl acetate. × 52,000.

PLATE 4

Fig. 8. A desmosome and myofibrillar insertion plaques from an intercalated disc in normal myocardium. Fixed in 2 % glutaraldehyde, post-osmicated and section-stained with lead citrate and uranyl acetate. × 95,000.

Figs. 9 and 10. Quintuple-layered membrane junctions in longitudinal sections of intercalated discs from normal myocardium. In fig. 9, the membranes are parallel to the electron beam and they are slightly inclined to the beam in fig. 10. At *L.* in fig. 9, the central element of the quintuple-layered junction appears as a line, while lower in the field a row of dots can be seen. Fixed in 10 % formalin, post-osmicated and section-stained with potassium permanganate. × 110,000.

Fig. 11. Quintuple-layered membrane junction in a transverse section of normal myocardium. Fixed in 1 % osmium tetroxide and section-stained with potassium permanganate. × 200,000.

Plate 5

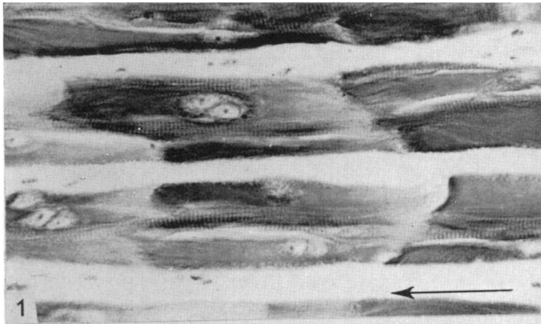
Figs. 12 and 13. Longitudinal sections of portions of the intercalated discs between two Purkinje fibres. Fixed by immersion in 1 % osmium tetroxide and section-stained with potassium permanganate. Fig. 12, × 40,000; fig. 13, × 100,000

Plate 6

Fig. 14. Dissociated normal cardiac muscle cells from the ventricles of the rat heart. Unfixed and mounted in glycerol. Phase contrast. × 290.

Fig. 15. As fig. 14. Fixed in 5 % formalin and stained haematoxylin and eosin. × 200.

Figs. 16 and 17. Longitudinal sections of the ends of dissociated cardiac muscle cells. Fixed in 1 % osmium tetroxide and section-stained with potassium permanganate. Fig. 16, × 12,800; Fig. 17, × 112,000.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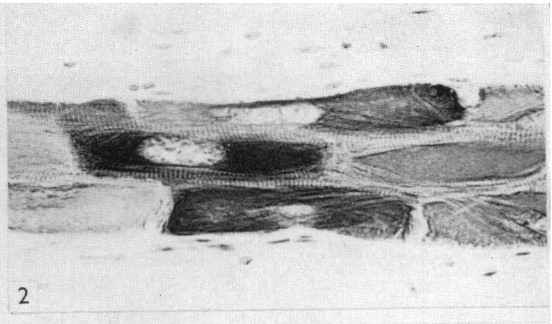
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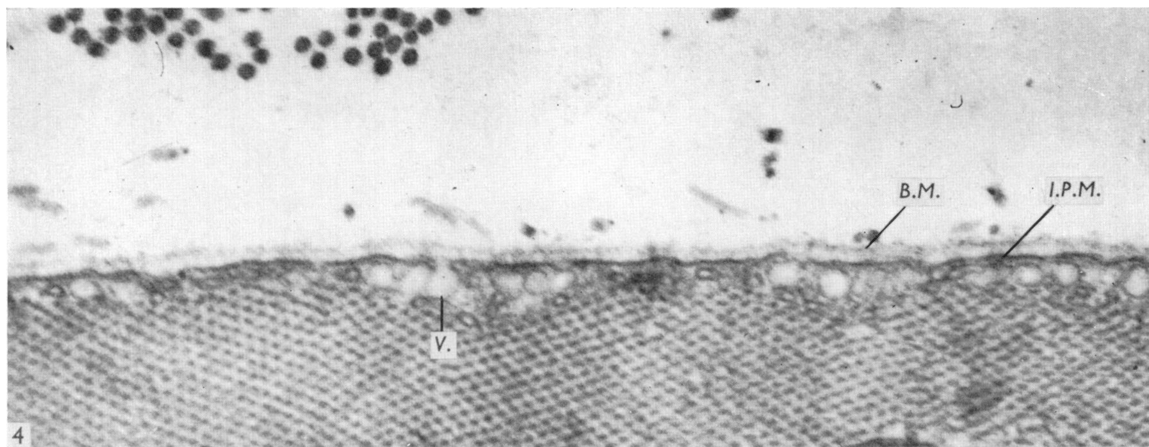
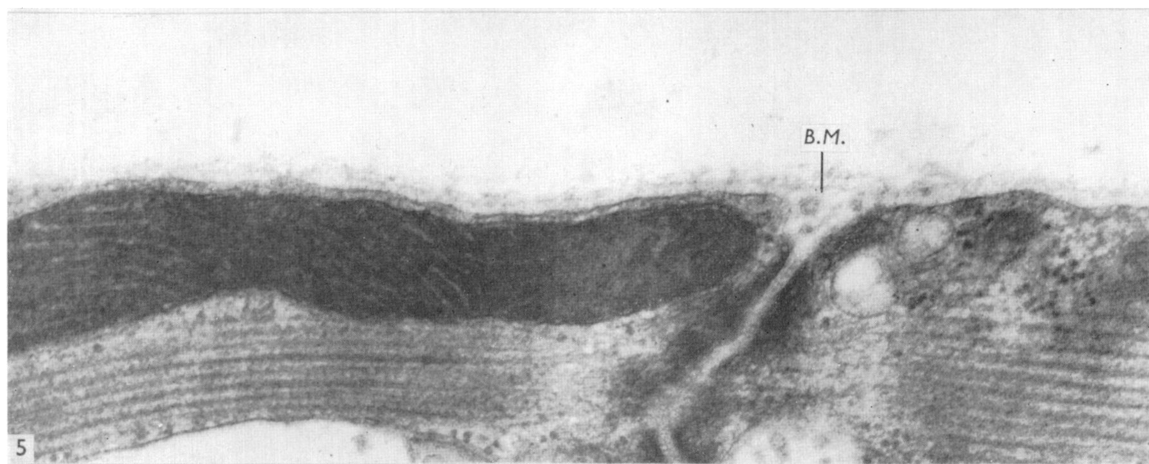
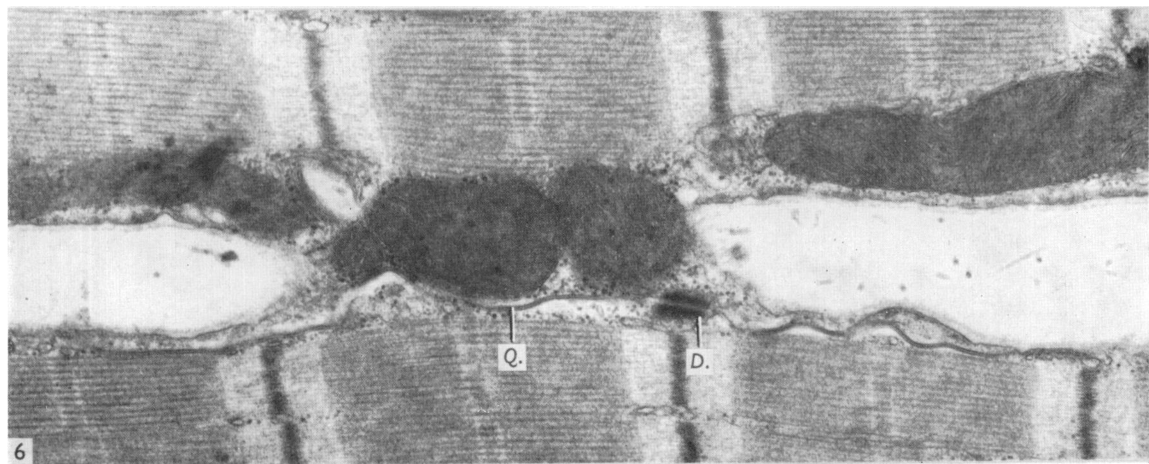
Plate 2







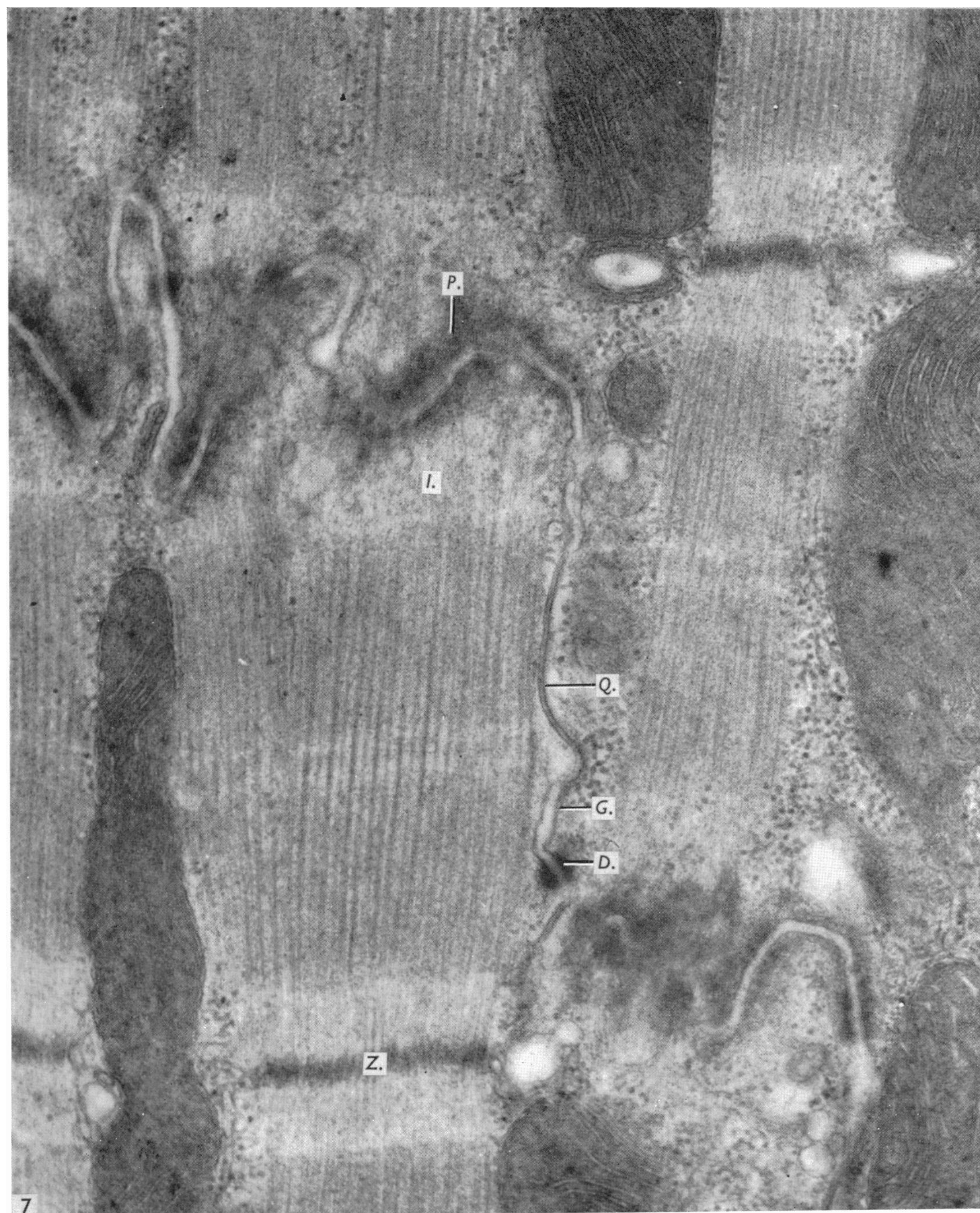
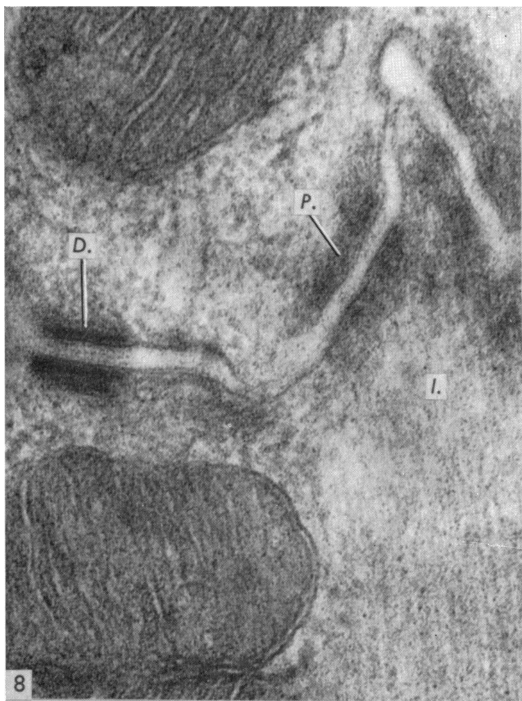
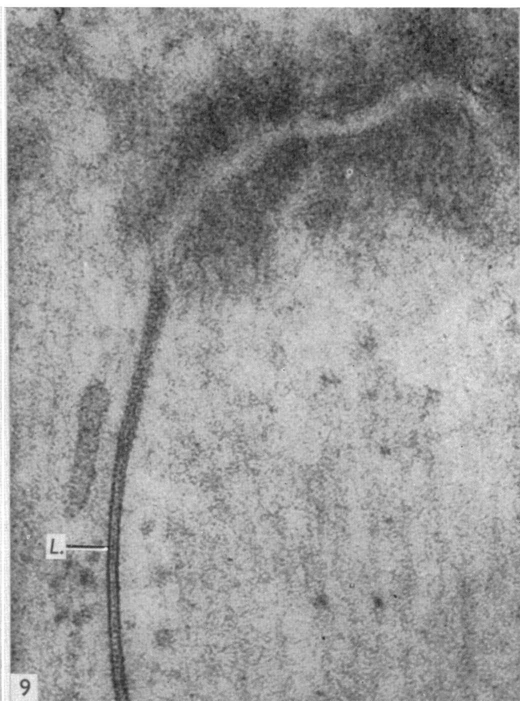
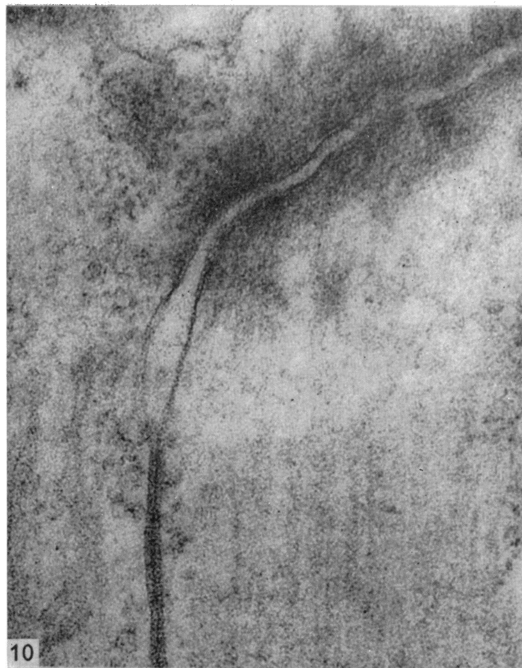
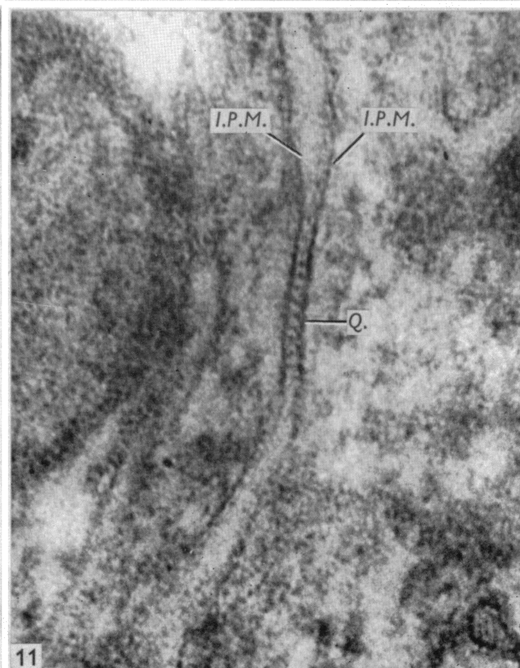

P.
I.
Q.
G.
D.
Z.
7

Plate 4









D.
P.
12

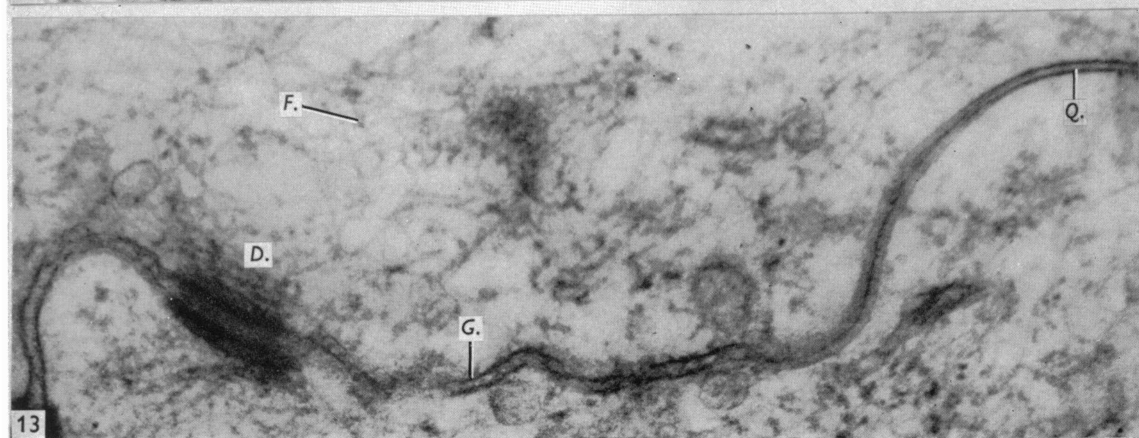


Plate 6