Virchows Arch. A Path. Anat. and Histol. 386, 43–58 (1980)

Virchows Archiv A

Unusual Familial Cardiomyopathy with Storage of Intermediate Filaments in the Cardiac Muscular Cells

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Summary. Unusual histological and ultrastructural changes in cardiac muscle cells have been found in 3 brothers with progressive myocardial deficiency. Histologically, this cardiomyopathy was characterized by massive storage of PAS-negative proteinaceous material in most cardiac muscle cells. The electron microscope showed that this material consisted of sinuous filaments, 7–10 nm in diameter, similar to the intermediate filaments normally present in cardiac muscle cells. Filament storage coincided with the disintegration of neighbouring myofibrils, with particular change in Z bands giving rise to rod-like bodies and more complex structures formed by the association of Z band material and sarcoplasmic reticulum (SR) tubules. Filament storage and myofibrillar disintegration always occurred in areas where the SR developed and involuted extensively. Relatively high glycogen accumulation also occurred, in close relation to the SR changes. Discrete SR proliferation, glycogen overload and filament deposits were observed in a few skeletal fibres.

These observations suggest that disturbance in the metabolism of desmin (protein subunit of intermediate filaments and a fundamental component of Z bands) might be involved in this type of cardiomyopathy. The influence of a chronic defect in calcium regulation might also be envisaged in view of the marked SR abnormalities.

Key words: Cardiomyopathy – Intermediate filaments – Sarcoplasmic reticulum – Glycogen

Introduction

Non-glycogenic material stored in muscular cells is generally in the form of basophilic, PAS-positive masses resembling Lafora's bodies, which are probably

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Acknowledgements. We are indebted to Georgette Haller and to Bernard Peltre for their invaluable technical assistance

0340-1227/80/0386/0043/$03.20

connected with disturbance of glycogen metabolism (see Rosai and Lascano, 1970; Ewing and Rosai, 1974). The present study concerns a cardiomyopathy affecting 3 brothers, and which is histologically characterized by the accumulation of non-basophilic, PAS-negative proteinaceous material in most of the cardiac fibres. Under the electron microscope, this material is seen to consist mainly of sinuous filaments, 7–10 nm in diameter, similar to the intermediate filaments which are a normal cytoskeletal component of heart-muscle cells. In addition to filament storage, other striking abnormalities in cardiac cells were observed, affecting the myofibrils and the sarcoplasmic reticulum (SR) in particular. Such cardiomyopathy, with storage of filaments in the myocardium, which appear as proteinaceous inclusions under the light microscope, has been rarely observed (Takatsu et al., 1968: idiopathic cardiomyopathy in a 22 year-old man; Sakakibara et al., 1970: idiopathic postpartum cardiomyopathy in a 30-year old woman); in neither case was there any cardiac abnormality in the other members of the families.

Case Reports

3 brothers, K.R. born in 1939, K.M. in 1948 and K.J. in 1954 were admitted to hospital in 1962, 1977 and 1978 respectively for severe cardiomyopathy with a complete auriculo-ventricular block needing the implantation of a pace-maker. Detailed clinical and biological data on these cases have already been published (Sacrez et al., 1979). Cardiomegaly was considerable in the 3 cases. Echocardiography revealed, in K.M., concentric hypertrophy, marked thickening of both the left ventricular wall and the septum, and restriction of the left ventricular cavity; and in K.J., an obstructive hyperthrophy with a particularly marked thickening of the septum. K.M. and K.J. had been known for some years to have heart trouble. K.R. died of heart insufficiency in 1973. No signs of cardiomyopathy were detected in the parents. A fourth brother, born in 1946, has no obvious heart trouble.

Material and Methods

In the case of K.R., only a post mortem histological examination was carried out. For K.M. and K.J., biopsies of myocardium (left ventricle) and of intercostal muscle were performed when the pace-makers were implanted. Biopsies of the deltoid muscle were made later. Part of each sample was fixed in formalin and paraffinembedded for routine histopathological examination. For electron microscopy, fragments were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4., some were post-fixed with 1% osmium tetroxide, in the same buffer, and all were embedded in an araldite-epon mixture. Semithin sections of this material were stained for light microscopic examination either directly, with toluidine blue in 5% Na borate, or with various routine techniques after removal of the embedding medium with Na methoxide (Mayor et al., 1961). Thin sections, contrasted with uranyl acetate and lead citrate, were examined under a Siemens Elmiskop IA.

Results

Light Microscopy

Large areas, bereft of myofibrils, consisting of an amorphous PAS-negative substance with no clearcut affinity for basic or acidic stains are seen in most cardiac fibres (Fig. 1 a–d). On paraffin sections, these areas are slightly stained with histochemical reactions for proteins (ninhydrin-schiff, coupled tetrazonium, dinitrofluorobenzene). On the semithin, PAS-hematoxylin-stained sections, glycogen appears mainly concentrated around the amorphous areas inside which it can also de detected in smaller amounts. Scattered granules stained with ferric hematoxylin, probably myofibrillar remnants, are also observed within these areas. Amorphous areas are frequently surrounded by a layer of tightly packed fine clear vacuoles which can be also detected among the amorphous material. In some fibres, clusters of large vacuoles are seen in close relation to amorphous areas. When stained with toluidine blue without removing the embedding medium with Na methoxide, these formations appear osmiophilic and strongly basophilic (Fig. 1 d). The nuclei are conspicuously enlarged and frequently contain glycogen. A few fibres contain voluminous bodies almost filling their cross-section and formed by the agglomeration of masses which are metachromatically stained with toluidine blue and intensely stained with alcian blue and PAS (even after glycogen digestion with amylase) (Fig. 1 e, f). These formations are identical to those described as "basophilic (mucoid) degeneration", "acid mucopolysaccharide storage", etc. and often reported as being like Lafora's bodies.

In skeletal muscle fibres, no alterations could be detected with light microscopy, apart from a mainly marginal glycogen loading in some fibres.

Electron Microscopy

Areas which appear amorphous under the light microscope consist mainly, sometimes exclusively, of accumulations of sinuous filaments, 7–10 nm in diameter (Figs. 2–6). These filaments are either grouped in bundles or, more often, randomly oriented and intermingled, forming a more or less dense network (Figs. 3, 4, 6). At the periphery of filament clusters, specific relationships between filaments and myofibrils can be observed. Isolated myofibrils are sheathed by whorls of filaments, tufts of which are linked to Z bands (Fig. 5). Superficial filaments are also seen to be fixed to dense plaques (hemidesmosomes) along the sarcolemma. These filaments are probably identical to those described in the normal myocardium, mainly in the impulse-conducting system (Virágh and Challice, 1969; Virágh and Porte, 1973) and in the force-producing fibres embedded in connective tissue (Virágh and Challice, 1969; Virágh and Törö, 1971) which are seen interlinking the Z bands and attached to dense plaques resembling hemidesmosomes or half zonulae adherentes along the sarcolemma facing the

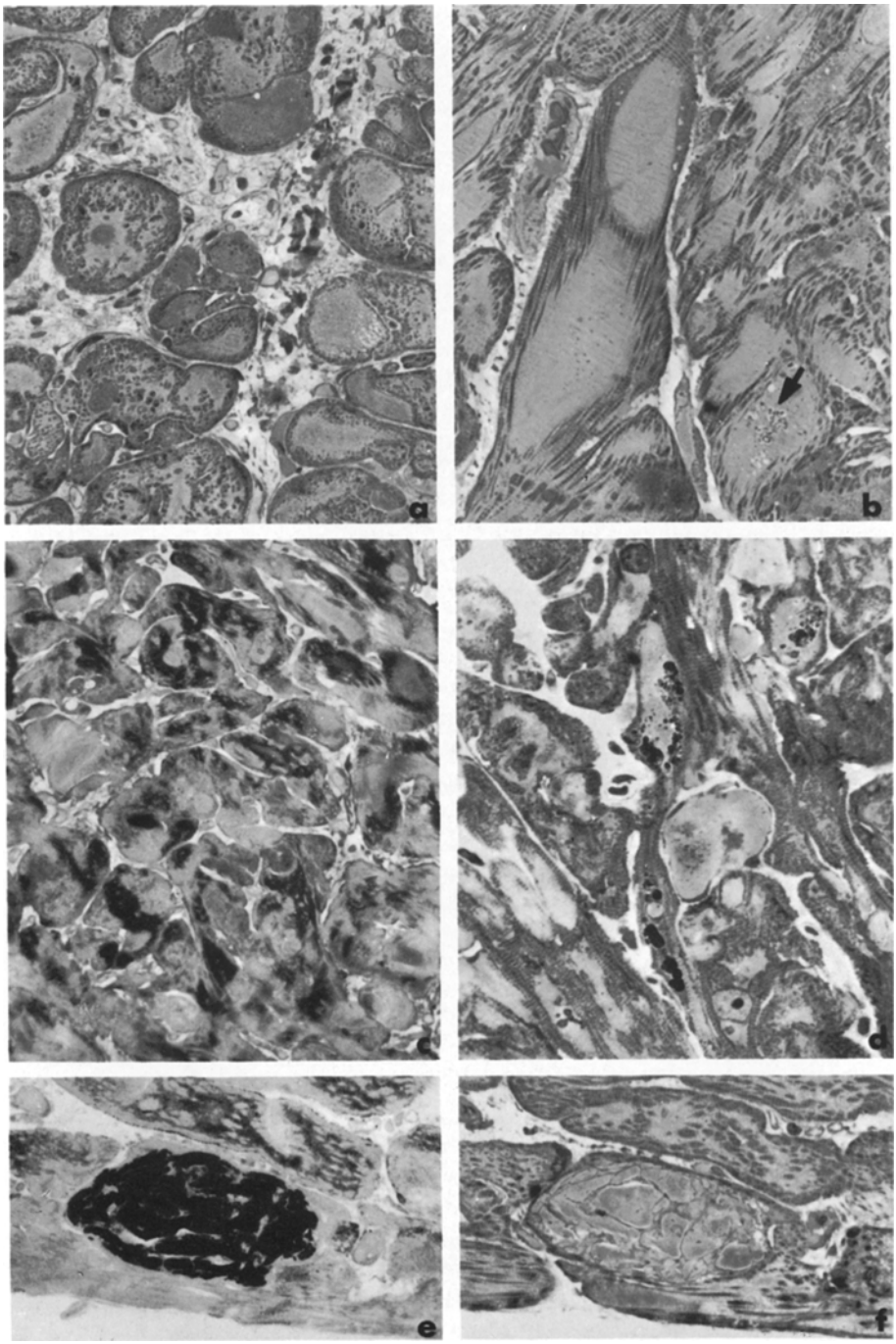

Fig. 1a–f. Semithin sections from cardiac biopsies from K.J. (**a, e, f**) and K.M. (**b, c, d**). **a** and **b**. Large amorphous areas devoid of myofibrils in most cardiac cells. Note, in **b**, clusters of clear vesicles stemming from partially extracted osmiophilic material (*arrow*). Ferric hematoxylin-eosin. **a** × 300; **b** × 450. **c**. Amorphous areas are not stained with PAS which does reveal glycogen accumulation around them. × 380. **d**. Clusters of basophilic osmiophilic bodies in some cardiac cells. Section stained with toluidin blue without removal of the embedding medium. × 450. **e** and **f**. Consecutive sections of an inclusion resembling Lafora's body, formed by an aggregate of strongly PAS-positive masses (**e**), less intensely stained with ferric hematoxylin (**f**). × 400

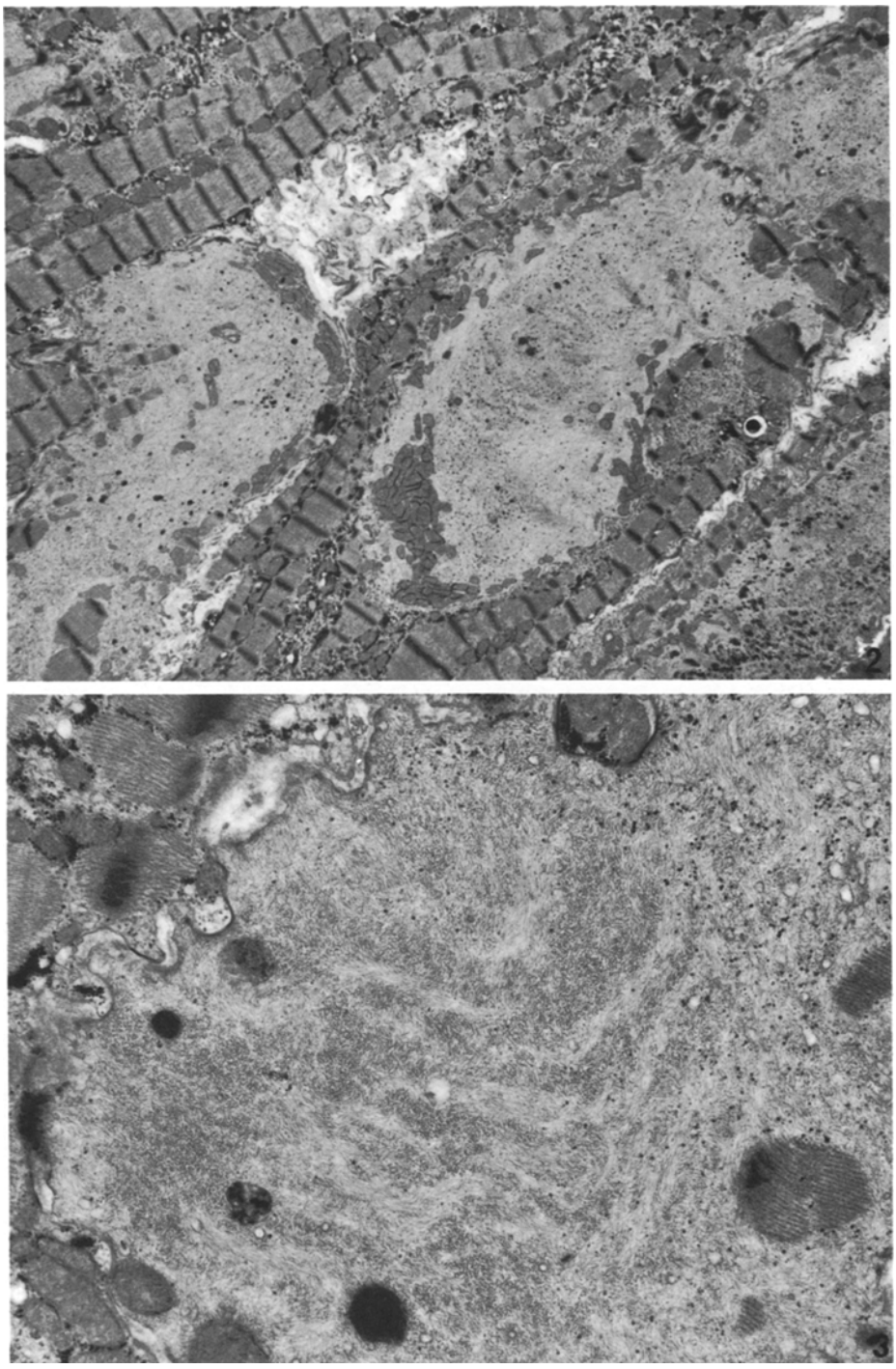

Fig. 2. General aspect of filamentous areas; note peripherally-packed mitochondria. Case K.M. $\times 2{,}000$

Fig. 3. Close to an intercalated disc (*left*), filamentous area formed by interwoven bundles of filaments which, in the peripheral zone (*right*), are intermingled with SR elements and glycogen particles. Case K.M. $\times 18{,}000$

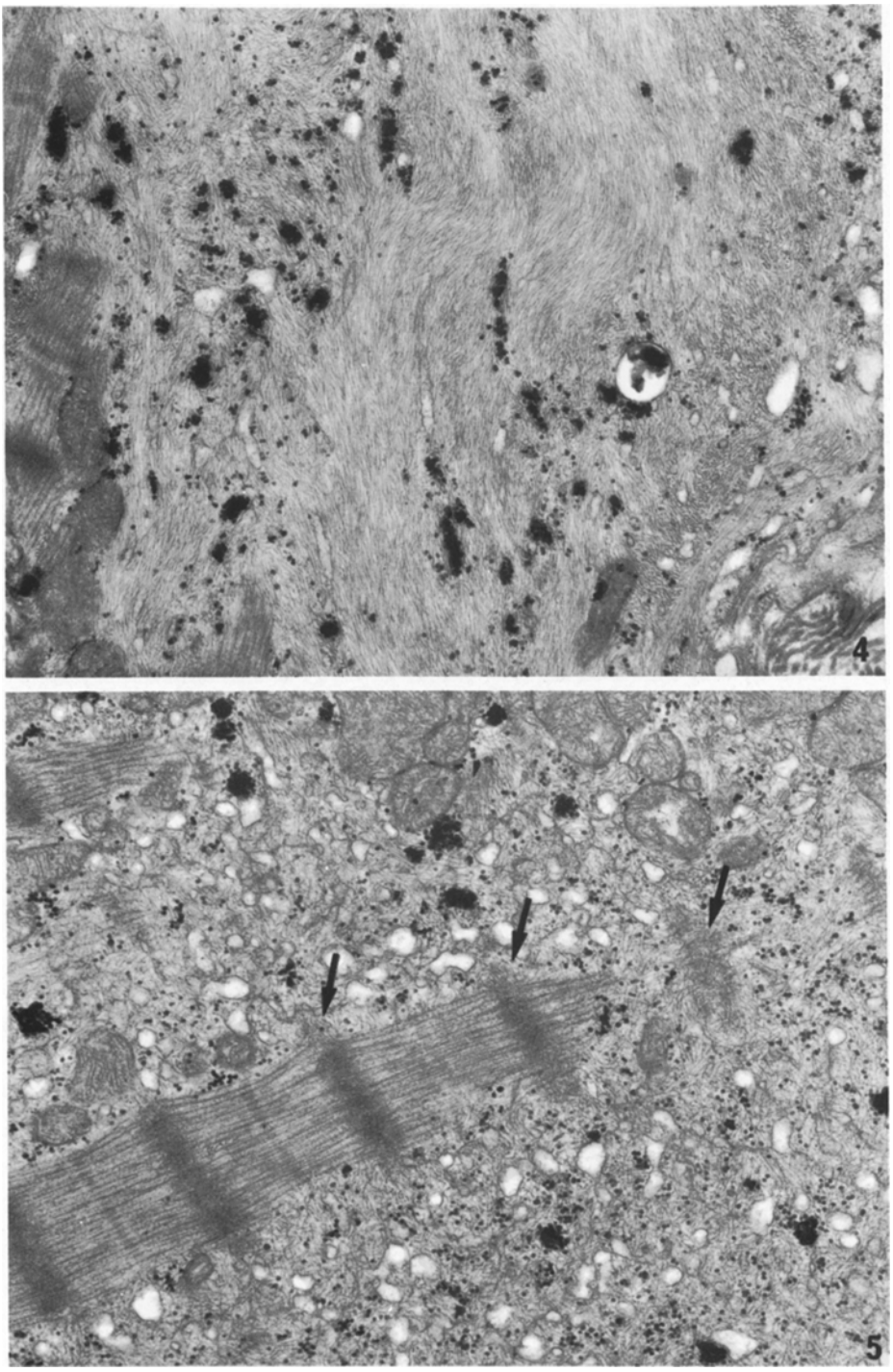

Fig. 4. Filamentous area containing numerous aggregated glycogen particles and a few SR tubules dispersed among packed filaments. Case K.M. ×21,500

Fig. 5. Randomly oriented filaments intermingled with abundant SR elements and glycogen particles; note tufts of filaments connected with the Z bands of an isolated myofibril (*arrows*). Case K.J. ×22,800

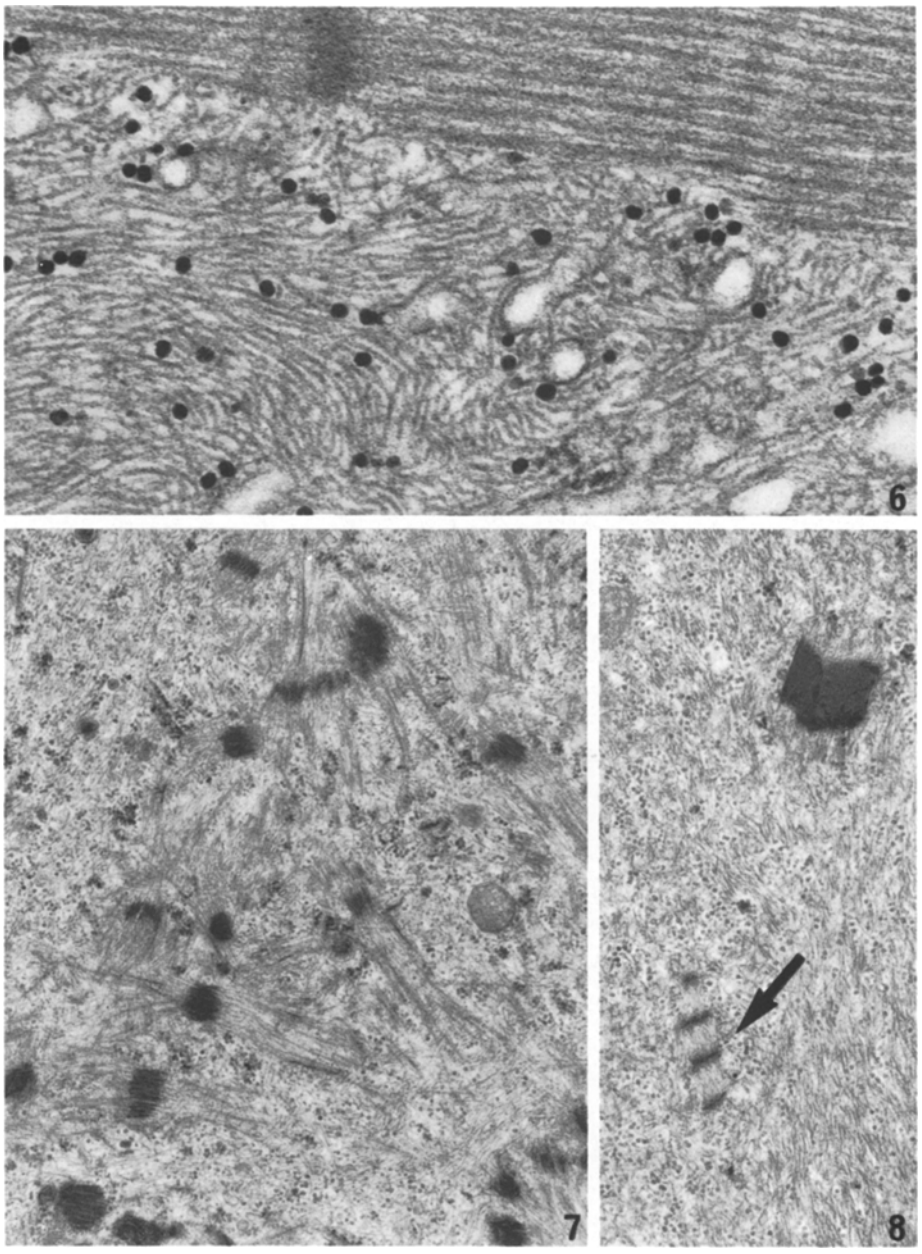

Fig. 6. Higher magnification showing the shape of the accumulated intermediate filaments; at the top: a myofibril. Case K.M. ×66,000

Fig. 7. Area of myofibrillar disorganization and break down, with Z band condensation. case K.M. ×21,600

Fig. 8. Isolated leptofibril still present among the accumulated filaments (*arrow*). Upper right, rod-like body. Case K.M. ×25,500

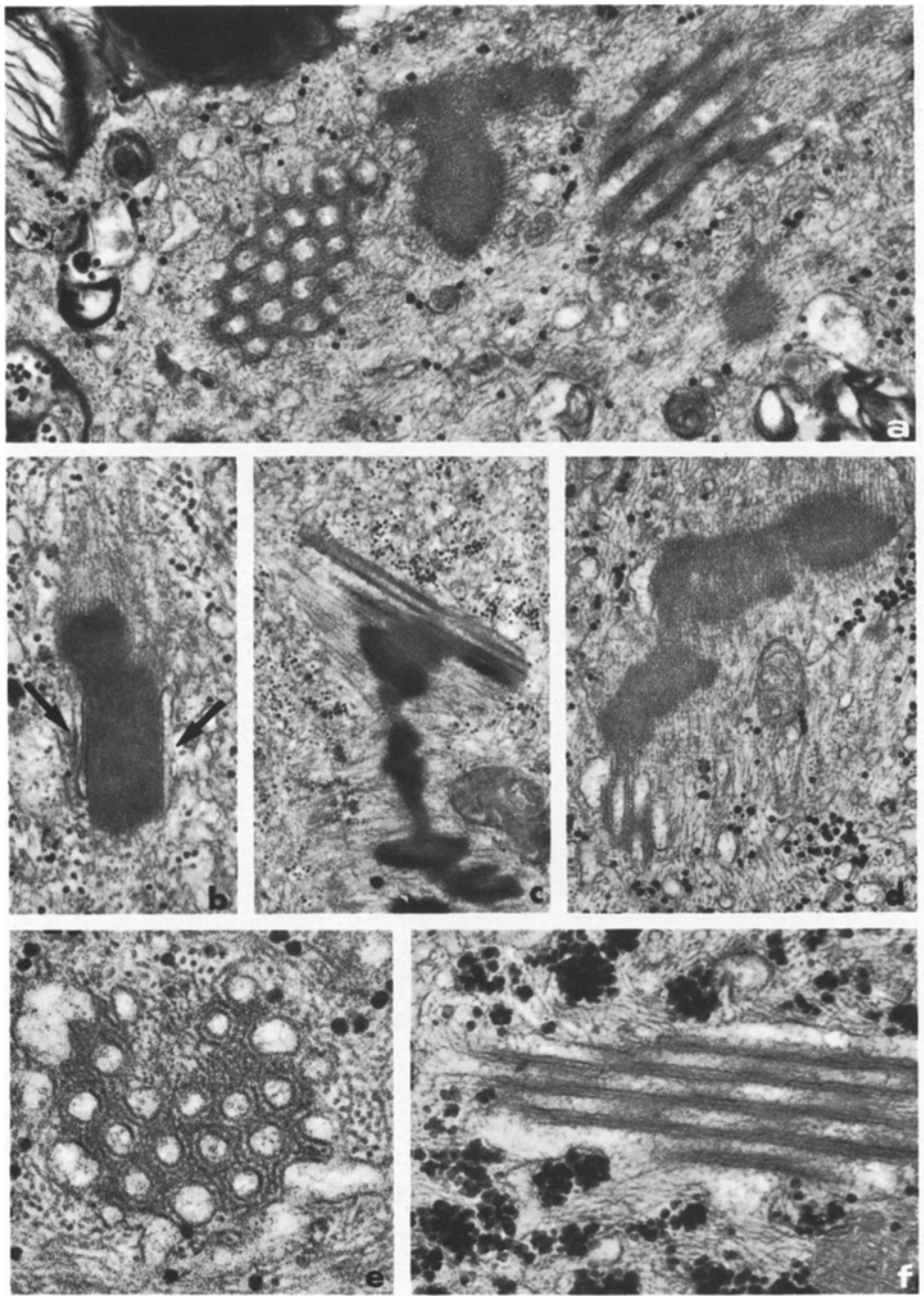

Fig. 9a–f. Different aspects of the rod-like and the "honeycomb" bodies: **a** "honeycomb" formations in transverse (*left*) and oblique (*right*) sections on both sides of a rod-like body. Note part of a large myelin body (*upper left*). ×38,000. **b** SR tubules (*arrows*) closely apposed on both sides of a rod-like body. ×30,000. **c** and **d** Straight parallel SR tubules in the lateral zones of rod-like bodies, seen on longitudinal (**c**) and oblique (**d**) sections. Continuity between dense filaments forming the rod-like body and the lamellar sheets surrounding the SR tubules is obvious on **d**. **c** ×19,200; **d** ×30,000. **e** Transversal section showing anastomosed lamellar sheets surrounding the regularly disposed SR tubules. ×67,000. **f** The lamellar sheets appear as continuous lines on longitudinal sections. ×40,500

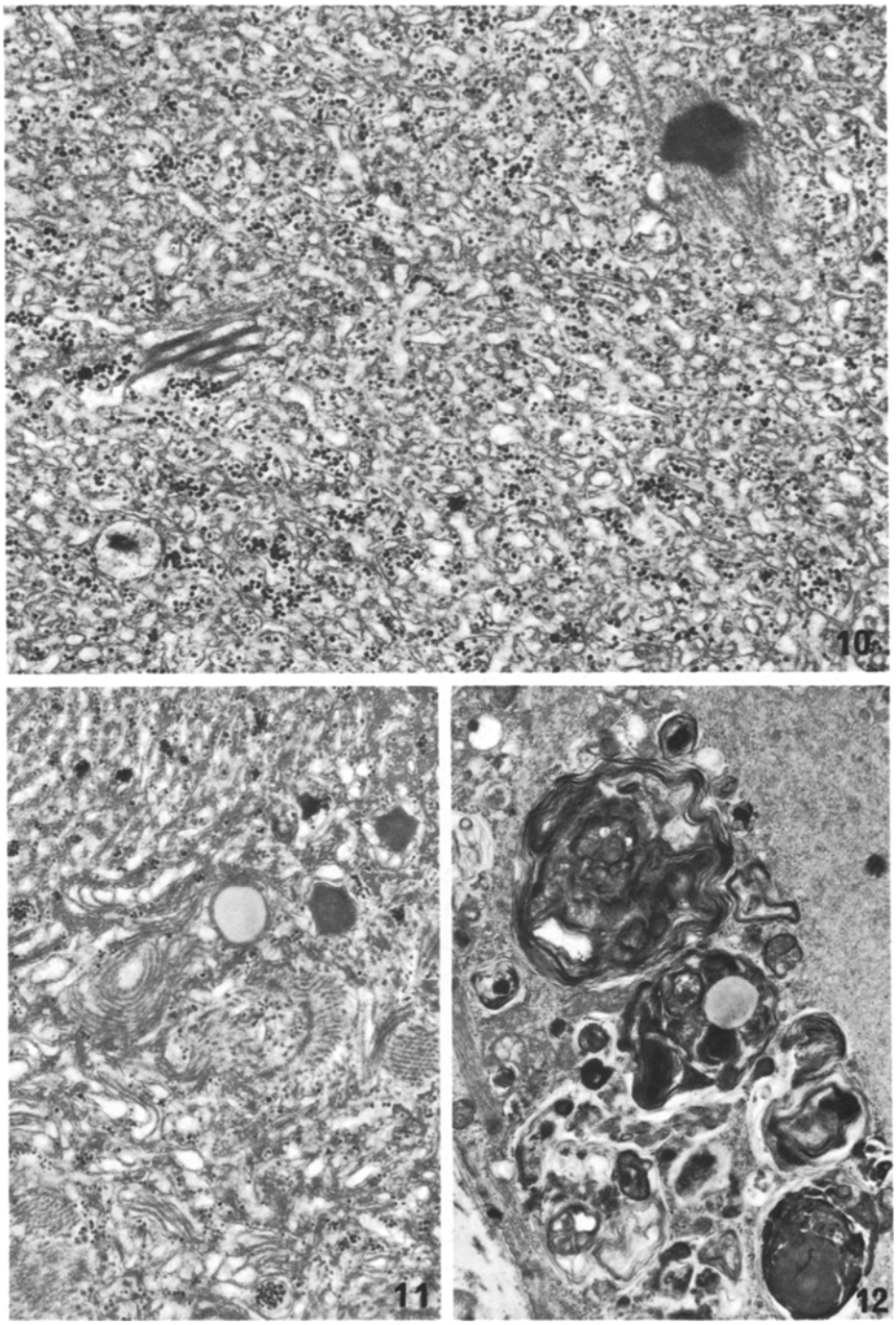

Fig. 10. Extensive accumulation of SR elements associated with glycogen particles. Note the presence of a "honeycomb" body (*left*) and of a rod-like formation (*right*). Case K.M. ×25,000

Fig. 11. Area of SR proliferation where curling up and coalescence of membranes indicate incipient SR involution. Note rod-like bodies with closely apposed SR tubules. Case K.M. ×21,600

Fig. 12. Involution features of the SR resulting in the accumulation of myelin bodies of various sizes, some of which condense into amorphous osmiophilic masses, in contact with a filamentous area. Case K.M. ×10,000

connective tissue. This class of filaments (intermediate filaments) is now well characterized as desmin filaments (Lazarides and Hubbard, 1976, 1978) in muscle cells.

Disintegration of myofibrils is frequently observed in contact with filament clusters (Fig. 7). Myofibrillar disruption coincides with structural alterations in the Z bands which result in formations resembling the "rod-like bodies" formed from Z bands in various muscle diseases (see review in Fardeau, 1970). These dense bodies (Figs. 8, 9), still linked with thin myofilaments, are scattered in the filamentous material and probably correspond to the small granules stained with ferric hematoxylin on semithin sections. On longitudinal sections, they seem to be formed by the close apposition of dense and straight filaments in continuity with thin myofilaments. Oblique sections show lattice arrangements of tightly packed and interconnected dense filaments. A paracrystalline aspect can sometimes be seen in transversal sections. Unlike the rod-like bodies generally described, there are no obvious periodic transverse striations on longitudinal sections. These bodies are often associated with "honeycomb" structures (Fig. 9) composed of straight parallel SR tubules surrounded by a fine dense lamellar layer in continuity with the dense filament network of the "rod" bodies. SR elements are frequently in close apposition with rod-like bodies (Fig. 9b) and can be seen penetrating them. Around these penetrating SR tubules, the dense filament network appears to condense into thin sheats which, on longitudinal and transverse sections, appear as continuous lamellae (Fig. 9: d, e, f). On transverse sections, the sheets of neighbouring SR tubules are seen to anastomose, forming a wide-meshed lattice (Fig. 9e). SR tubule walls are typically stiff when enclosed in the lattice. This rigidity of both the SR tubules and their lattice sheets is clearly visible on longitudinal sections (Fig. 9c, f).

Accumulations of "Z band material" similar to those described in this work have been reported in degenerating cardiac muscle cells in patients with cardiac hypertrophy (see review in Maron and Ferrans, 1978) and comparable associations between "Z band material" and SR tubules have also been seen in some of these cells, where SR development was particularly marked (Maron and Ferrans, 1974, 1978). In the type of cardiomyopathy that we report here, accumulations of "Z band material" coinciding with myofibrillar disintegration are regularly found in the cardiac muscular cells containing filament deposits, i.e. in almost all the cardiac muscular cells studied.

Apart from these bodies formed from Z bands of disintegrated myofibrils, small periodically striated bundles of thin filaments, known as leptomeric fibrils

Fig. 13. Filamentous area, rich in glycogen particles, which tend to aggregate around myelinic bodies. Case K.M. ×21,600

Fig. 14. Marginal zone of a formation resembling Lafora's body. Progressive transition of peripherally aggegated glycogen particles into the dense amorphous and fine fibrillar material. Case K.J. ×32,500

Fig. 15. Accumulation of mitochondria with dense intra-matrix inclusions. Case K.M. ×23,800

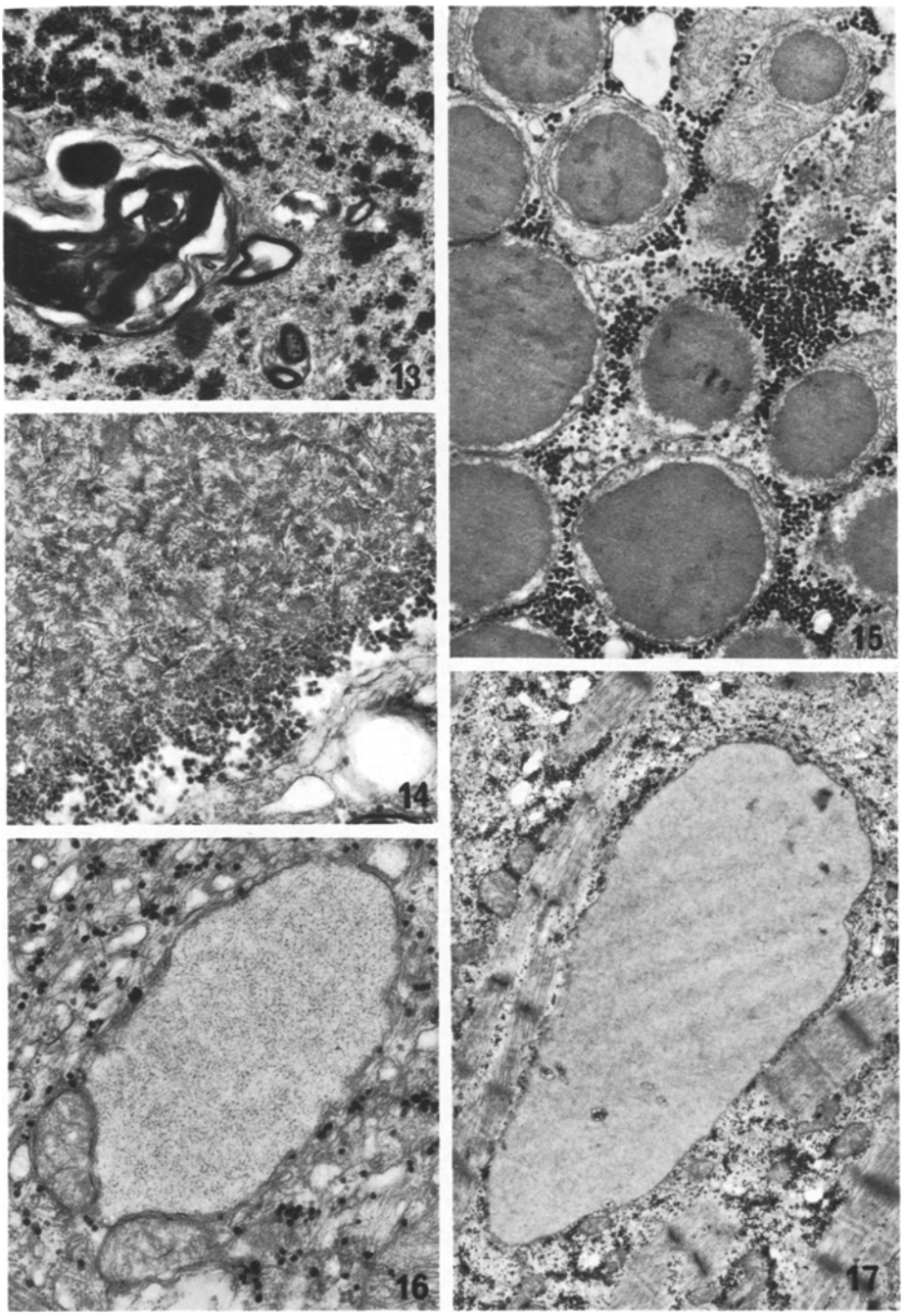

Fig. 16. Vacuole with low-density content, in close contact with mitochondria, in an area rich in S.R. Case K.J. ×35,000

Fig. 17. Very large vacuole, with amorphous content, not obviously related to any organelle. Case K.M. ×8,000

(Thoenes and Ruska, 1960), which are normally attached to the Z bands, are also observed singly in the filamentous areas (Fig. 8).

Glycogen particles are always present in the filamentous areas. They can be abundant, forming voluminous clusters at the periphery of the filamentous areas. Small aggregates of glycogen particles surrounded by incurved, dilated SR tubules and small autophagic bodies containing aggregated glycogen particles can be found. This non-specific glycogen resorption process is not, however, very marked.

In some areas with less abundant filaments the SR, in contrast, is conspicuously developed (Figs. 5, 10). Glycogen particles are always closely associated with the SR. Certain areas poor in filaments are largely filled with these reticulo-glycogenic complexes (Fig. 10). In such areas, involuton of the SR is often observed, sometimes affecting most of the SR tubules (Fig. 11). Curling up and coalescence of membranes gives rise to myelin bodies which condense progressively into strongly osmiophilic masses (Figs. 12, 13). These membraneous bodies, grouped in large clusters, can be clearly identified by their intense basophilia on semithin sections stained with toluidine blue, without removing the embedding medium (Fig. 1 d). They are sometimes associated with fine lipid droplets, a typical feature of lipopigments. SR involution is both massive and extensive in some cardiac cells with accumulations of numerous myelin bodies. Varying quantities of intermediate filaments are always found intermingled with the SR elements, and myofibrillar disruption is seen in these areas, as at the periphery of the large clusters of filaments.

Mitochondria are often tightly packed at the periphery of filamentous areas (Fig. 2), but are normally distributed in the remainder of the cardiac muscle cells. Mitochondria with spherical osmiophilic intramatricial inclusions are often observed; this non-specific modification (see Fardeau, 1970) can affect a noticeable proportion of the mitochondrial population in some cells (Fig. 15). Various sized cavities lined by a single membrane and filled with an amorphous or vaguely reticulated weakly electron-dense substance are occasionnally observed (Figs. 16, 17). These structures are sometimes in close contact with mitochondria (Fig. 16). They are mainly localized in the marginal cytoplasm and can be seen apposed to the plasma membrane. The largest cavities can be detected with the light microscope on semithin sections where they show slight metachromatic staining with toluidin blue. The origin of these vacuoles is not clear. They resemble smaller cavities with similar content which can be seen to originate from dilation of terminal cisternae of the SR in skeletal muscle in various muscular diseases (unpublished observations).

The nuclei have jagged outlines, due to deep-branched cytoplasmic invaginations which often contain glycogen (Fig. 18). The extensive development of cytoplasmic intranuclear areas contributes largely to the apparent increase in nuclear size. Comparable nuclear abnormalities have been reported in hyperthrophied human cardiac cells (Ferrans et al., 1975; Maron and Ferrans, 1978). These modifications are particularly frequent and extremely marked in this type of cardiomyopathy.

The large, confluent, basophilic and intensely PAS-positive masses seen in a few cardiac cells under the light microscope (Fig. 1 e, f) appear as aggregates

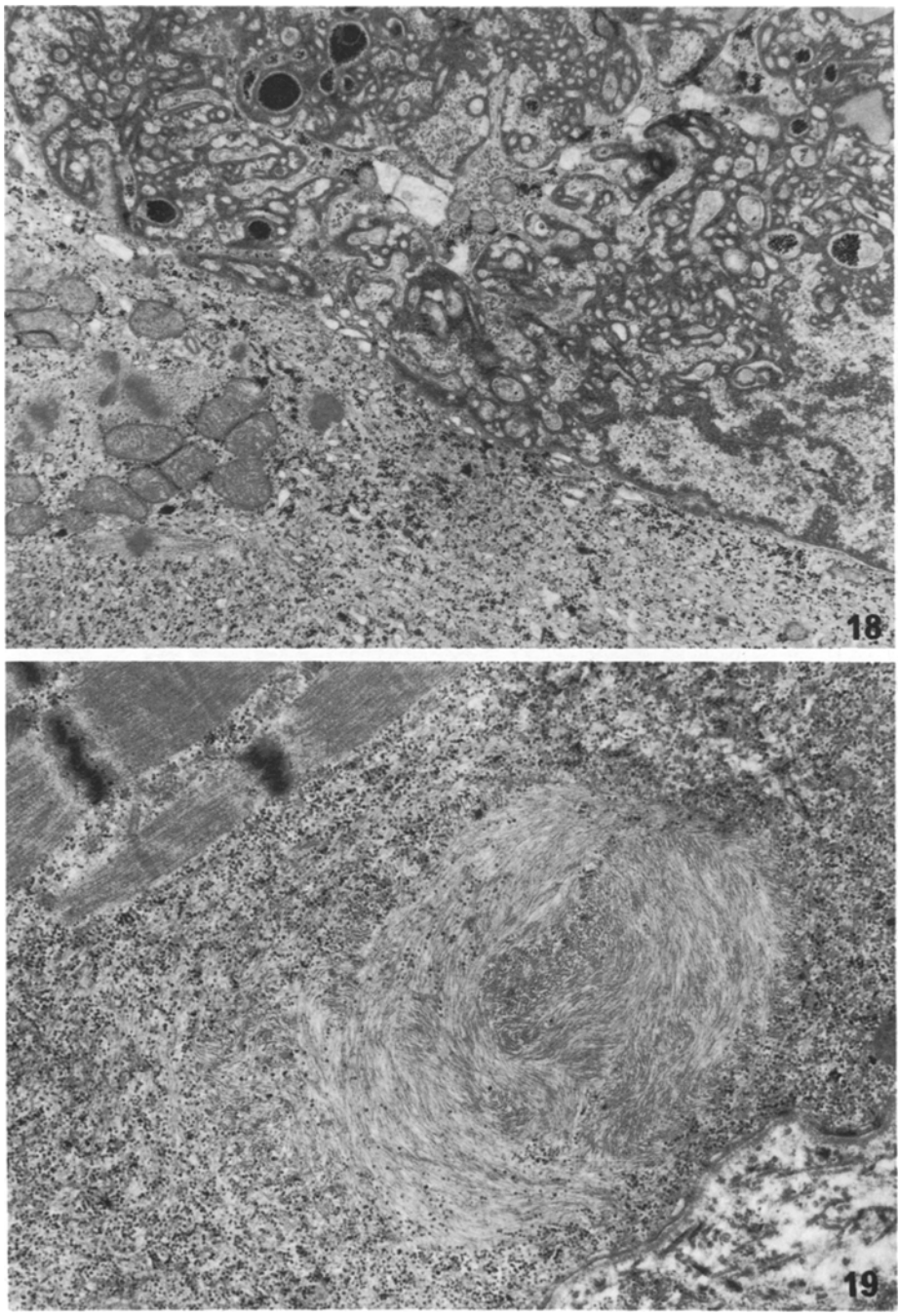

Fig. 18. Characteristic modification in nuclear outlines: extensive digitated cytoplasmic invaginations, some of which contain glycogen particles. Case K.M. ×12,000

Fig. 19. Skeleatal muscle fibre with marginal accumulation of intermediate filaments and glycogen particles. Note the scattered and whorled arrangement of the filaments. Case K.M. ×18,000

of fine fibrillary structures embedded in a strongly electron-dense amorphous substance. The peripheral layers of these masses consist of tightly packed glycogen particles (Fig. 14). These formations occur in glycogen storage areas; the glycogen particles seem to be progressively replaced by fibrillar and amorphous components which, like glycogen, stain intensely with the silver proteinate technique (Thiery, 1966) and are not contrasted with lead citrate after "en bloc" uranyl acetate staining. This latter technique does not, however, prevent staining with Thiery's method. These bodies have the same ultrastructural and histochemical properties as Lafora's bodies and similar inclusions reported in various conditions in the nervous and muscular systems (see Powell et al., 1978). They are considered to be accumulations of polyglucosans bound to acid (mainly phosphate) groups, resulting from incomplete glycogen hydrolysis (Rosai and Lascano, 1970; Martin et al., 1973; Powell et al., 1978).

In skeletal muscle, a more developed SR and relative overload of glycogen particles is observed, especially in marginal areas of fibres where dispersed or clustered intermediate (7–10 nm) filaments are seen (Fig. 19). Filament deposits are generally discrete, no traces of them being visible under the light microscope. The SR sometimes presents involutionary features, consisting of a few dispersed myelin bodies. No other obvious alterations have been observed in muscle fibres.

Discussion

Accumulations of intermediate filaments in cardiac muscular cells, visible under the light microscope as large proteinaceous inclusions, have rarely been reported in cardiomyopathies; they were interpreted either as abnormal protein deposits (Sakakibara et al., 1970) or as a muscular degradation process (Takatsu et al., 1968). In these cases, the same cardiac changes were observed in the auricular and ventricular walls. Histological post mortem examination in the first case (K.R.), which was reported as cardiomyopathy with "protein" storage (Batzenschlager, 1974), showed that these changes affected the whole myocardium diffusely. This cardiac disease appears well characterized by both its histological and ultrastructural features. A genetic factor is probably involved, since the same cardiac changes were found in three brothers.

Intermediate filaments are well defined cytoskeletal structures in muscle cells. Their protein subunit "squelettin" (Small and Sobieszek, 1977) or "desmin" (Lazarides and Hubbard, 1976, 1978) of around 50,000 MW can polymerize to filamentous or non-filamentous forms, and electively bind actin. Desmin protein appears as a fundamental component of the Z band, where it may serve to integrate the terminating sarcomeric actin filaments, and might also link actin filaments to plasma membrane at the fasciae adhaerentes of the intercalated discs (Lazarides and Hubbard, 1978). Desmin and desmin filaments by providing lateral connections between the Z bands of individual myofibrils might be involved in uniting the contractile action of the myofibrils (Lazarides and Hubbard, 1978). These data on desmin enable correlations to be envisaged between the myofibrillary changes, particularly affecting the Z bands, and the storage

of intermediate filaments. A disorder of desmin metabolism could thus explain the most significant changes observed in the cardiac muscle cells in this type of cardiomyopathy.

Filamentous storage and myofibrillar disrupture coincide with conspicuous SR changes and clearly occur in areas where the SR develops and involutes. Various stages of this process can be seen in different cells, and even within one single cell. The modifications in the SR suggest that a disturbance in the regulation of intracytoplasmic calcium movements might be involved in this myocardial disease, possibly acting on the desmin-actin relationship and the polymerization of the desmin subunits.

Glycogen changes, i.e. glycogen particle accumulation linked with SR development, glycogen resorption features and rare formations resembling Lafora's bodies are far from specific and do not lead one to envisage any chronic defect in the glycogenolysis pathway. Furthermore, enzymological data revealed normal phosphorylase, amylo 1.6. glucosidase and acid α glucosidase activities in heart and skeletal muscle biopsies in K.M. and K.J. (De Barsy; for details of the biochemical studies see Sacrez et al., 1979).

In skeletal muscle, SR proliferation and glycogen accumulation are confined to small marginal areas where small clusters of intermediate filaments are also present, suggesting that desmin metabolism in the skeletal fibres might be perturbed to some extent. These modifications reflect only faintly the major cardiac changes. Electromyographic examination revealed only slight abnormalities in the 3 cases.

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Accepted October 12, 1979