Myocardial Ultrastructure in Idiopathic Hypertrophic Subaortic Stenosis

A Study of Operatively Excised Left Ventricular Outflow Tract Muscle in 14 Patients

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SUMMARY

Electron microscopic studies revealed distinctive abnormalities in operatively resected myocardium from the left ventricular outflow tract in 14 patients with idiopathic hypertrophic subaortic stenosis. Bundles of muscle cells were severely disorganized, with cells running in different directions instead of in parallel. Muscle cells were wider and shorter than in hypertrophy due to other causes and showed increased cellular branching, extensive side-to-side intercellular junctions, widened Z bands, and evidence of formation of new sarcomeres. Some myofibrils were oriented obliquely or perpendicular to the longitudinal axes of the cells and some myofilaments that originated from a single Z band inserted into Z bands of other myofibrils. Examination of left ventricular apical myocardium in two patients revealed hypertrophied but normally arranged muscle cells. It is concluded that abnormal architecture of muscle cells is the basic morphologic feature of idiopathic hypertrophic subaortic stenosis.

Additional Indexing Words:
Electron microscopy  Ventricular hypertrophy

IDIOPATHIC HYPERTROPHIC subaortic stenosis (IHSS, hypertrophic obstructive cardiomyopathy) is characterized grossly by asymmetric hypertrophy of the ventricular septum. A bizarre arrangement of the muscle cells in the asymmetrically hypertrophied ventricular septum was noted histologically by Teare in his necropsy study of 10 patients with this entity. Subsequently, several reports have been made of the ultrastructure of muscle cells in the ventricular septum in patients with IHSS. These studies, however, are in disagreement as to whether or not the myocardial hypertrophy observed in IHSS is different from that found in other cardiac diseases. An investigation of this problem forms the basis of the present communication, which describes observations on the ultrastructure of cardiac muscle resected at operation in 14 patients with well-documented IHSS.

Materials and Methods

Patients Studied

The 14 patients (six men and eight women; average age, 41 years) presented the usual clinical, angiographic, and hemodynamic findings (table 1) of IHSS. Five patients had small peak systolic pressure gradients (PSG) between the right ventricle and the pulmonary trunk. At rest, 11 patients had a PSG between the left ventricle and a systemic artery ranging between 20 and 180 mm Hg (average 76). One patient had a PSG of 5 mm Hg, and the remaining two patients had no gradient at rest; however, gradients from...
Table 1

Hemodynamic Data on Patients with Idiopathic Hypertrophic Subaortic Stenosis

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<th>No.</th>
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Abbreviations: RV = right ventricle; PA = pulmonary artery; PSG = peak systolic gradient; LV = left ventricle; SA = systemic artery; s/d = systolic/diastolic.

75 to 105 mm Hg (average 95) were provoked in these three patients either by the Valsalva maneuver or by administration of isoproterenol. Before operation all patients were in functional classes 3 or 4 (New York Heart Association), and all had received propranolol (80–400 mg/day, orally) in attempts to alleviate their symptoms.

Preparation of Tissues

Samples of muscle from the asymmetrically hypertrophied portion of the left side of the ventricular septum (14 patients) and from the subepicardial area of the apex of the left ventricle (two patients) were obtained for morphologic study at the time of left ventricular myotomy-myectomy. The tissues were fixed with cold 3% glutaraldehyde in 0.1M phosphate buffer, pH 7.2, washed with several changes of buffer, postfixed with 1% osmium tetroxide in 0.1M phosphate buffer, pH 7.2, dehydrated, and embedded in Maraglas.2 Semifine (0.5 μ thick) sections of these tissues were stained with alkaline toluidine blue and studied by light microscopy for selection of areas for electron microscopic examination. Tissues in which contraction bands or other artifacts of tissue preparation were present were excluded, and final choice of areas for electron microscopic study was limited to those which contained artifact-free, longitudinal sections of muscle cells. Ultrathin sections of these areas were cut with an LKB Ulrotome, stained with uranyl acetate and lead citrate, and examined with an RCA EMU-3G electron microscope.

Results

Electron microscopic study of tissues resected at operation from the upper portion of the left side of the ventricular septum revealed strikingly consistent abnormalities in all 14 patients. Such a consistent pattern of abnormal findings was remarkable in view of the wide variations in age and severity of left ventricular outflow obstruction in these patients. No correlation could be established between the extent of the abnormalities and age, sex, symptomatology, degree of elevation of right or left ventricular end-diastolic pressure, PSG, or with familial history of IHSS (patient no. 10 and possibly patients no. 2 and 12-14).

The most characteristic abnormality observed was disorganization in the arrangement of muscle cells, myofibrils, and myofilaments. The disorganization varied considerably in extent and severity from one area to another in each heart, but was observed in all. A detailed account of these abnormalities is given below, followed by descriptions of other changes found in the muscle cells. The structure of normal human myocardium is shown in figure 1 for purposes of comparison.
Abnormalities in Arrangement of Muscle Cells

Alterations in the arrangement of muscle cells were evident on light microscopic study of longitudinal sections of muscle cells in semithin sections stained with toluidine blue (fig. 2). Delineation of cellular boundaries was sometimes impossible in these preparations, due to the fact that the muscle cells often had extensive side-to-side intercellular junctions that could not be identified by light microscopy. The muscle cells were arranged in closely packed bundles, with small intercellular spaces and variable degrees of interstitial fibrosis. Most muscle cells were abnormally large, and many were also abnormal in shape. In some areas the hypertrophied muscle cells were rectangular in shape, ran parallel to each other, had end-to-end intercellular junctions (intercalated discs), and resembled those seen in cardiac hypertrophy due to causes other than I1HSS (fig. 2, top left). In other areas, many cells were shorter than normal and had transverse diameters of up to 80 μ; (normal, 10–15 μ); other cells had irregular shapes, with several branches that extended in various directions, so that each cell formed intercellular junctions with several others (fig. 2, top right and bottom left). The branching of these cells was reflected in the arrangement of their myofibrils, which coursed in several directions instead of following their normal orientation parallel to the longitudinal axis of the cell (fig. 2, bottom left). In some cells only a few myofibrils were abnormally oriented (fig. 2, bottom right).

Abnormalities of Myofibrillar Structure

Abnormalities of myofibrillar structure were localized in the Z bands and consisted of: widening of the Z bands (fig. 3); spreading of Z-band material toward the center of the sarcomere (fig. 4, top left and right); splitting of Z bands (fig. 4, bottom); attachment of Z bands to the sarcolemma; and the presence of increased amounts of material, similar to that
Figure 2

Light micrographs of semithin sections of myocardium from left ventricular outflow tract (LVOT) of patient no. 14. (Top, left) Group of hypertrophic muscle cells with normally
of Z bands, at points of attachment of myofibrils to intercellular junctions. These abnormalities varied in degree from one area to another and occurred in cells with normal myofibrillar orientation and in cells in which myofibrils and myofilaments were abnormally arranged.

Widening of Z bands was related to an increase in the amount of Z-band material in the sarcomeres rather than to abnormal degrees of sarcomere shortening. The widened Z bands appeared fibrillar at high magnification (fig. 4, bottom) and did not show the periodicity of about 200 Å that has been observed in other types of abnormal Z bands. The Z-band material often was distributed asymmetrically along the width of the Z band (fig. 3), especially in instances in which it was associated with abnormally oriented myofibrils. In some areas this material formed strands that extended toward the center of the sarcomere, often giving the impression that alterations of this type were related to the formation of new sarcomeres (fig. 4, top left and right). This concept was supported by the finding of actual splitting of Z bands (fig. 4, bottom).

The increase in Z-band material also was manifested by the frequent observation of accumulations of Z-band material in close contact with the sarcolemma and intercalated discs. Patches of Z-band material associated with the sarcolemma were found at the free surfaces of the cells, and in deeper areas, at the level of the transverse tubules (fig. 5, top). The extent of attachment of Z bands to transverse tubules varied considerably. Some widened Z bands spread out around the transverse tubules and attached to their sides, whereas others remained separated from the transverse tubules by narrow spaces. Attachments of Z bands to areas of the sarcolemma at the cell surfaces were observed in areas in which myofibrils were normally oriented; however, such attachments were most prominent in association with myofibrils that were oriented perpendicularly or obliquely to the longitudinal axis of the cell. Some of these peripherally located attachments also were strongly suggestive of the formation of new sarcomeres (fig. 5, bottom).

Abnormalities of Myofibrillar Orientation

The presence of branched cells with myofibrils oriented in various directions was evident by light microscopy (fig. 2). In addition, electron microscopic study showed that many unbranched cells also contained small areas with obliquely or transversely oriented myofibrils. It became evident that the pattern of myofibrillar organization ranged from entirely normal in some cells (fig. 5, top) to one of severe disarray in others (figs. 6 and 7) in which immediately adjacent myofibrils were sometimes oriented perpendicularly to each other. The latter pattern of arrangement was complicated by the fact that myofilaments that originated from a single Z band sometimes coursed in diverging directions and inserted into several different Z bands. Furthermore, some of these diverging filaments were arranged in a cross-weaving pattern (fig. 7). Such a pattern represented the most severe abnormality of myofibrillar structure.

When only a few myofibrils were affected, alterations of myofibrillar orientation were localized in the vicinity of the sarcolemma and intercalated discs. In subsarcolemmal areas (fig. 8), the abnormally oriented Z bands were wide and irregularly shaped, and sometimes they were continuous with immediately adjacent, normally oriented Z bands. These abnormally oriented Z bands usually occurred in small groups, and the myofibrils of which

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arranged myofibrils. (Magnification × 800.) (Top, right) Stellate-shaped muscle cell with myofibrils arranged in different directions. Cell outlines are indicated by arrowheads. (Magnification × 1000.) (Bottom, left) Two muscle cells have obliquely and transversely oriented myofibrils and a side-to-side intercellular junction (arrow). Note the wide transverse diameter (55 µ) of the cell in the center. (Magnification × 1000.) (Bottom, right) Muscle cell in which myofibrils are normally oriented except in area (arrow) at end of cell, where myofibrils are oriented transversely. (Magnification × 1200.)
Figure 3

Portion of two muscle cells from LVOT of patient no. 12, showing widened (Z₁) and abnormally arranged (Z₂) Z bands; attachment (A) of Z bands to plasma membranes; transversely oriented filaments (F) at the level of a Z band; transversely arranged myofibrils (MF); a side-to-side intercellular junction (IJ) with abnormally small desmosomes (D); and a clump of mitochondria (M). (Magnification × 17,600.)
they formed part followed circumferential paths along the transverse axes of the cells. Thus, the arrangement of these myofibrils resembled that of "Ringbinden"14 in skeletal muscle. By contrast, the disordered array of myofibrils was much more pronounced in areas near intercalated discs.

**Alterations in Other Cellular Components**

*Thin filaments* that measured 100 A in diameter were consistently associated with the Z bands. These filaments were oriented transversely, i.e., at right angles to the longitudinal axis of the myofibrils, formed parallel bundles, and connected adjacent Z bands with each other, thus bridging the gaps created between myofibrils by the presence of mitochondria and components of the triads (figs. 3 and 9). These filaments also connected Z bands with the outer nuclear membranes (fig. 10, top) and with the sarcolemma; they also were found frequently in the vicinity of intercalated discs (fig. 9). We have observed these filaments in normal myocardium in several animal species as well as in diseased human hearts (Unpublished observations). It is our impression, however, that these filaments were particularly numerous and prominent in the hearts of patients with IHSS.

The *nuclei* of the muscle cells were markedly enlarged and the nuclear membranes showed bizarre convolutions (fig. 10, middle). Some cells were binucleated. The degree of convolution of the nuclear membranes often appeared greater than could be accounted for on the basis of contraction of the muscle cells.

Many of the *intercellular junctions* (intercalated discs) were unusually convoluted and had large areas of side-to-side apposition instead of, or in addition to, end-to-end apposition (figs. 3 and 10, bottom). As mentioned previously, these changes were associated with condensations of Z-band-like material on the cytoplasmic aspect of the membranes of the discs. Such alterations appeared related to the irregular shapes of the cells and to the abnormal arrangement of their myofibrils. Separation of the membranes of the discs was not observed.

Some degree of *mitochondrial damage* was present in all hearts. This damage (minimal in seven patients, moderate in three, and severe in four) was characterized by swelling of mitochondria, disruption of cristae, and formation of intramitochondrial concentric lamellae that showed no periodicity (figs. 6 and 7). A marked increase in the numbers of mitochondria was observed in only one heart, in which this change was focal and primarily evident in perinuclear areas. In the other 13 hearts the numbers of mitochondria were comparable to those in cardiac hypertrophy due to causes other than IHSS.

The content of *glycogen* varied greatly from one cell to another in each patient. Artifactual changes in glycogen were observed in association with irregular contraction of the myofibrils, which resulted in displacement of glycogen granules toward less contracted areas of the cells. A marked and unequivocal, but focal, increase in glycogen was observed in one patient.

*Ribosomes* were present in increased numbers in most cells. They occurred free and in the perinuclear areas (fig. 10, top) and between myofibrils (fig. 9), as well as attached to the membranes of the sarcoplasmic reticulum (figs. 10, middle, and 11). The *sarcoplasmic reticulum* was normal in most areas, and dilatation of the tubules was seldom seen. The *transverse tubular system* was normal in five hearts and showed minimal to moderate dilatation in nine. *Lysosomes* and *lipofuscin* granules (fig. 11) were increased in number, particularly in other patients. *Lipid droplets* were rarely present in the muscle cells.

Variable numbers of collagenous fibers separating bundles of muscle cells were observed in the *interstitium*. Most capillaries were normal and *neural elements* were not abnormally large or numerous.

**Observations on Left Ventricular Apical Myocardium**

Myocardium from the subepicardial area of the apex of the left ventricle was examined in two patients. In both patients the apical muscle cells differed from those in the
asymmetrically hypertrophied portion of the ventricular septum. The apical cells were hypertrophied but were much more uniform in size, shape, and arrangement than those in the septum. In most apical cells the myofibrils were arranged parallel to the longitudinal axis of the cell (fig. 12, top). Minor degrees of divergence of myofibrils and widened Z bands were observed in some instances (fig. 12, bottom), but no transversely oriented myofibrils were found in apical cells. Intercellular junctions of apical cells occurred mostly end-to-end in the usual fashion of normal left ventricular myocardium. Changes in other cellular organelles resembled those observed in septal myocardium.

Discussion

The ultrastructure of the myocardium in IHSS has been the subject of several previous studies.2-8 In ventricular septal myocardium of five patients with this disease, Pearse2 observed abnormally large numbers of mitochondria, increased amounts of glycogen, and fragmentation and loss of myofibrils; however, he did not describe myofibrillar disorientation. Although Pearse also found increased numbers of sympathetic nerve fibers, others4,7 have been unable to confirm this observation, and reexamination by Van Noorden, Olsen, and Pearse8 of the material studied by Pearse2 led to the conclusion that connective-tissue elements were responsible for the fluorescence attributed by Pearse to the presence of catecholamines in nerve fibers. McCallister and Brown4 studied septal myocardium from eight patients with IHSS and observed disorganization of myofibrils in addition to the changes previously described by Pearse. They observed similar abnormalities in left ventricular apical muscle in two patients. McCallister and Brown concluded that the type of hypertrophy in IHSS differed from that in right ventricular myocardium in four patients with congenital heart disease, in which they found similar increases in mitochondria and glycogen but no disorganization of myofibrils. Meesen and Poche6, 6 studied the ultrastructure of septal myocardium in five patients with IHSS and observed that the muscle cells were arranged in disorderly bundles, coursed in several directions, contained increased numbers of mitochondria, ribosomes, and glycogen particles, and showed nuclear enlargement, increased cellular branching, increased areas of side-to-side intercellular junctions, and evidence of formation of new myofilaments in subsarcolemmal areas. These features are very similar to those found in the present study. In 10 patients with IHSS, Sonnenblick5 observed nuclear alterations, increased numbers of mitochondria, and variations in the length of sarcomeres within a given cell. In our opinion these variations in sarcomere length represent contraction bands. Snijder, De Jong, and Meijer2 studied myocardial ultrastructure in two patients with IHSS; in one patient the muscle cells contained large numbers of mitochondria and myofibrils that were wavy but otherwise normal; in the other patient the myofibrils were abnormally slender, wide apart, and often appeared interrupted and somewhat moth-eaten. Snijder, De Jong, and Meijer concluded that the microscopic lesions in IHSS were of a nonspecific nature. A similar conclusion was reached by Van Noorden, Olsen, and Pearse8 who compared histochemical and electron microscopic findings in septal myocardium from 17 patients with IHSS (including the five patients previously studied by Pearse), three patients in whom the diagnosis of IHSS was in doubt, and 33 patients with a variety of other cardiac

Figure 4

Abnormalities of Z bands in IHSS. (Top, left) Irregular spreading of Z-band material toward center of sarcomere. LVOT of patient no. 12. (Magnification × 29,500.) (Top, right) Strands of Z-band material extend throughout sarcomere in myofibril that is out of register with adjacent one. LVOT of patient no. 10. (Magnification × 15,900.) (Bottom) Asymmetrical splitting of Z band suggestive of formation of new sarcomere. LVOT of patient no. 10. (Magnification × 32,000.)
Figure 5

Abnormalities of Z bands in LVOT muscle in IHSS. (Top) Z bands show mild, irregular widening and attachment to membranes of T tubules (T) and to sarcolemma (S) in cell in which myofibrils are normally arranged. Patient no. 2. (Magnification × 13,000.) (Bottom) Small Z band (Z₁) is present just under sarcomere (S), and is located at the level of the middle of the sarcomere limited by Z bands Z₁ and Z₂. Two sets of myofilaments (one between Z₁ and Z₂, the other between Z₁ and Z₃) are present between Z₂ and Z₃ in the area in which...
MYOCARDIAL ULTRASTRUCTURE

disorders. By light microscopy, Van Noorden, Olsen, and Pearse observed that in IHSS the bundles of muscle cells were frequently arranged in a disorderly fashion and often formed whorls in which the cells were oriented in a concentric, ringlike arrangement; by electron microscopy they observed only changes similar to those described by Pearse. The 33 hearts with other types of heart disease showed ultrastructural changes similar to, but less severe than, those in IHSS. Although they showed that disorganization of muscle bundles seldom occurred in the 33 control hearts, Van Noorden, Olsen, and Pearse concluded that the ultrastructural changes considered to be typical of IHSS were probably characteristic of extreme hypertrophy due to diverse causes.

The results of our study are in agreement with previous observations that the muscle cells in IHSS are arranged in irregular bundles1, 3, 6, 8, 15–18 are frequently branched,3–6 and have increased areas of side-to-side intercellular contact.3, 6 Our observations also confirm and extend the data of McCallister and Brown,4 who noted that the contractile elements also are abnormally arranged. The observations of McCallister and Brown4 and of Snijder et al.7 were not published in full, for which reason they cannot be compared in detail with ours. Although Pearse6 and Van Noorden, Olsen, and Pearse8 did not demonstrate abnormalities of myofibrillar orientation in IHSS, their published electron micrographs appear to have been taken from normally arranged cells, rather than from whorls of cells such as those shown in their light micrographs. We found that myofibrillar abnormalities occurred much more frequently in cells in the latter arrangement. Although we recognize the variable extent to which myofibrillar abnormalities are present in different areas of tissue, it has been our experience that these changes are difficult to recognize by electron microscopy unless longitudinal sections of muscle cells are studied in close correlation with light microscopic observations of sections from the same tissue block. We believe, therefore, that these factors account for the differences between our observations and those of others.2, 8

A distinction between nonspecific changes and those which appear unique to IHSS is necessary for a detailed analysis of the data presented in this study. The specificity of these changes can be evaluated only within the context of our limited knowledge of the ultrastructure of human myocardium in various other diseases. The patients in the present study had very severe IHSS and underwent operation because of their poor response to medical management. These patients, therefore, represent the most severe form of the disease rather than its whole clinical spectrum.

We consider that changes in numbers of mitochondria, glycogen, and ribosomes, alterations in nuclear size and shape, dilatation of the transverse tubular system, and widening of Z bands are nonspecific findings related to hypertrophy of muscle cells. Only the alterations in the shape of the muscle cells and the abnormalities of myofibrillar structure and orientation appear unique to IHSS.

Changes in Glycogen

Increased amounts of glycogen, particularly in perinuclear areas, have been observed consistently2–6, 8 in cardiac muscle cells in IHSS. We have observed these changes in our patients with IHSS, and also in patients with other types of cardiomyopathies (Unpublished data). Large accumulations of glycogen appear to form in perinuclear areas of hypertrophied cardiac muscle cells because of changes induced by hypertrophy in the geometry of these regions of the cell. The myofibrils normally diverge and converge again as they course around the nucleus,

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Z_1 \text{ occurs, but only one set of myofilaments is found between } Z_2 \text{ and } Z_3 \text{ in the area of the sarcomere just below } Z_1. \text{ This arrangement is interpreted as indicating that a new } Z \text{ band (} Z_n \text{) is developing, along with two sets of myofilaments, on the subsarcolemmal side of the sarcomere bounded by } Z_2 \text{ and } Z_3. \text{ Note the free ribosomes (R) and glycogen particles (C). Patient no. 4. (Magnification } \times 21,000.\text{)}
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Circulation, Volume XLV, April 1972
Area of muscle cell in vicinity of intercalated disc (ID) shows several areas of myofibrillar disarray (arrows), widened Z bands, and mitochondrial damage. LVOT of patient no. 13. (Magnification × 6900).
Figure 7

Area of marked disorganization of myofibrils and myofilaments in muscle cell from LVOT of patient no. 2. A cross-woven arrangement of myofilaments is evident in several areas (arrows), and myofilaments that originate from single Z bands (Z) insert into several different myofibrils that course in various directions. (Magnification × 21,900.)

Circulation, Volume XLV, April 1972
Abnormalities of myofibrillar orientation in LVOT muscle in IHSS of patient no. 12. (Top) Periphery of muscle cell showing longitudinal section of superficial myofibrils oriented perpendicular to those deeper in cell. (Magnification × 14,750.) (Bottom) Same as above, but showing cross section of superficial myofibrils and longitudinal section of deeper myofibrils. (Magnification × 25,500.)

Figure 8

Circulation, Volume XLV, April 1972
Figure 9

High magnification view of side-to-side junction between two muscle cells, showing cross sections of bundles of transversely oriented filaments (F) at the level of Z bands and in the vicinity of the attachment of a widened Z band to the plasma membrane. LVOT of patient no. 2. (Magnification × 52,000.)
leaving a conical area, which is filled with mitochondria, glycogen, lipofuscin granules, and the Golgi complex, at each nuclear pole. When the nucleus enlarges and displaces adjacent myofibrils to the sides of the cell, the conical areas at the nuclear poles increase in size and thus can contain more mitochondria and glycogen. In addition, contraction of muscle cells at the time of biopsy tends to displace mitochondria and glycogen toward myofibril-free areas such as the perinuclear zones, thereby causing a further increase in their content of mitochondria and glycogen. For these reasons we believe that perinuclear accumulations of glycogen and mitochondria are nonspecific changes of cardiac hypertrophy. Nevertheless, we observed a marked, focal increase in glycogen in one of our patients; the etiology of this change is unknown. Some patients with type II glycogenosis (Pompe's disease) have left ventricular outflow tract obstruction, due to massive enlargement of muscle cells filled with glycogen deposits; however, there is no evidence to suggest that IHSS is related in any way to a disturbance in glycogen metabolism.

Changes in Mitochondria

It is clear that the hypertrophic muscle cells in IHSS often contain large numbers of mitochondria. It is our impression, however, that comparable numbers of mitochondria are present in IHSS and in cardiac hypertrophy of other causes. The mitochondrial damage and intramitochondrial concentric lamellae observed in this study are of uncertain etiology. These changes may have been related to longstanding hypertrophy and failure, or to elective cardiac arrest during operation. It is also possible that they were caused or worsened by the administration of propranolol, which has been reported to produce similar alterations in normal mouse myocardium. All of our patients had received propranolol.

The increased numbers of ribosomes also represent a nonspecific change seen in other types of cardiac hypertrophy. This alteration is undoubtedly related to the increase in protein synthesis that mediates the process of hypertrophy. The increase in nuclear size and irregularity of nuclear shape and the dilatation of the transverse tubular system have been observed in other conditions and also appear to be nonspecific changes of cardiac hypertrophy. The lack of fatty change and abnormalities in the sarcoplasmic reticulum is surprising since these changes are frequently seen in other cardiomyopathies.

Widening of Z Bands

Structural changes in Z bands have attracted considerable attention recently because of their relationship to the formation of new sarcomeres in cells undergoing either normal growth or hypertrophy. These alterations are characterized by various degrees of widening of Z bands, and can be classified into two types, depending on whether or not the affected Z bands show a repeating periodicity of 175–200 Å along the transverse axis of the myofibril. As discussed below, the significance of this periodicity is uncertain, and both types of Z-band alterations have been described in similar conditions.

Widening of Z bands without associated periodicity has been observed in sarcoid heart disease, in early phases of hypertrophy after experimental aortic insufficiency, in rheumatic heart disease, in atria with hypertrophy due to a variety of causes, in normal atrioventricular node and bundle of His.

Figure 10

(Top) View of part of the nucleus of a muscle cell showing filaments (F) connecting Z bands of myofibrils to nuclear membranes (NM). Note the en face view of nuclear pores (NP). LVOT of patient no. 14. (Magnification × 34,000.) (Middle) Longitudinal section of muscle cell showing bizarre-shaped nucleus. LVOT of patient no. 9. (Magnification × 11,250.) (Bottom) Extensive side-to-side intercellular junction (II) between two muscle cells. Area on left is shown at higher magnification in figure 9. LVOT of patient no. 2. (Magnification × 13,500.)
Perinuclear area of cardiac muscle showing large numbers of ribosomes (R) attached to membranes of cisterns of reticulum, glycogen particles (G), several lysosomes (LY) that contain lipid droplets and highly organized material, and part of a lipofuscin granule (LG). LVOT of patient no. 4. (Magnification × 47,250.)
Figure 12

Longitudinal sections of left ventricular apical muscle cells from patient no. 13. (Top) Myofibrillar orientation and sarcomere alignment are normal. (Magnification × 3900.) (Bottom) Some myofibrils show minor degrees of divergence from axial orientation. (Magnification × 5100.)
and, as shown by this study, in IHSS. Widened Z bands with the periodic structure described above were first observed by Shy et al.\textsuperscript{33} in skeletal muscle of patients with a congenital “nemaline” type of myopathy, but are now known to occur in several other skeletal muscle disorders.\textsuperscript{34} They have been described in normal ventricular myocardium and right bundle branch of monkeys\textsuperscript{31} and in myocardium of old but apparently normal cats\textsuperscript{10} and dogs;\textsuperscript{11} in the latter, however, only some of the widened Z bands showed periodicity. Fawcett\textsuperscript{10} pointed out the similarity between the periodic arrangement of these Z bands and that observed in crystals of tropomyosin, a normal component of Z bands. Fawcett concluded that these widened Z bands were a consequence of excessive production of Z-band material, probably tropomyosin, and that they resulted from the rearrangement and side-to-side association of Z-band filaments to form broad, cross-striated Z bands. Bishop and Cole\textsuperscript{12} found widened Z bands, similar to those described by Fawcett\textsuperscript{10} in hypertrophied and failing right ventricular myocardium of four dogs (three with pulmonary arterial banding, one with congenital valvular pulmonic stenosis), and concluded that the altered Z bands played a role in the formation of new sarcomeres and elongation of myofibrils. Côté, Mohiuddin, and Roy\textsuperscript{30} observed Z-band widening, without periodicity, in 24% of 57 atrial appendages of patients with various congenital and acquired cardiac lesions and found a correlation between Z-band widening and degree of atrial hypertrophy.

Legato\textsuperscript{13} reported Z-band widening, with somewhat variable patterns of periodicity, in myocardium from 19 patients with congenital heart disease (15 samples of right atrium, two of right ventricle, and three of left ventricle), in tissue culture cells grown from newborn rat ventricle, and in all four cardiac chambers of newborn puppies. In all these tissues she observed the presence of excessive amounts of Z-band material, particularly in peripheral areas of the cells, i.e., just under the sarcolemma and at intercellular junctions. This distribution resembled that observed by us in patients with IHSS. Legato concluded that these changes were related to the formation of new sarcomeres and postulated that the Z bands may serve as the template upon which new contractile elements are laid down in developing or enlarging cardiac muscle cells. Legato pointed out that tropomyosin exists as a highly hydrated gel and that variations in techniques of tissue dehydration during preparation for electron microscopy may cause profound changes upon its structure. Côté, Mohiuddin, and Roy attributed the lack of periodicity in widened Z bands in their material to the fact that they used glutaraldehyde as a primary fixative, rather than osmium tetroxide as did others who observed this periodicity;\textsuperscript{10-12} however, Viragh and Chalice\textsuperscript{31} and Legato\textsuperscript{13} demonstrated this periodicity in tissues fixed with glutaraldehyde. Additional study will be necessary to determine whether the preceding variations in the substructure of widened Z bands are related to methods of specimen preparation or to differences in chemical composition. The latter possibility is suggested by the fact that we have observed periodicity in widened Z bands in glutaraldehyde-fixed human skeletal muscle (Unpublished observations) but not in glutaraldehyde-fixed myocardium from patients with IHSS.

The transversely oriented filaments found at the levels of the Z bands in myocardium of patients with IHSS constitute a population of filaments that differ from those which constitute the myofibrils. These filaments appear to form a cytoskeleton that attaches myofibrils to each other, to the nuclear membranes, and to the sarcolemma. These filaments have been described previously in adult myocardium of monkey,\textsuperscript{31} rat, and rabbit.\textsuperscript{35} The present report documents for the first time the attachment of myofibrils to the nuclear membranes. Filaments of the same diameter (about 100 Å) as those described above are present in developing cardiac\textsuperscript{36-38} and skeletal\textsuperscript{39,40} muscle cells, where they are randomly oriented and are known as “intermediate filaments” because their diameter is between

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that of the thin and thick filaments (60–80 Å and 130–150 Å, respectively) of the myofibrils. The exact relationship of these intermediate filaments to those in adult myocardium remains to be determined. In agreement with Virág and Chalice31 we believe that the transversely oriented filaments at the level of Z bands function in maintaining sarcomeres in register. Our observations also suggest that these filaments are responsible for the changes in nuclear shape that occur41, 42 during contraction and relaxation of cardiac muscle cells.

Abnormalities of Myofibrillar Orientation

Considerable evidence indicates that myofibril formation during normal development of cardiac muscle occurs mainly in subsarcolemmal areas of the cells.18, 43 This process has a similar localization in human cardiac muscle in the usual type of hypertrophy13 and in IHSS. Nevertheless, formation of new sarcomeres in the common types of cardiac hypertrophy13 is not associated with the abnormal myofibrillar orientation that we have observed in IHSS. Obliquely and transversely oriented myofibrils such as those in IHSS have been observed in regenerating cardiac muscle cells at the periphery of areas of infarction44, 45 and are found normally to a limited extent in cells of the A-V node and bundle of His.31, 32 A disorderly arrangement of myofibrils is characteristic of embryonic cardiac muscle, in which myofibrils in early stages of formation have myofilaments that radiate in several directions from a single Z band.46 Branching of myofibrils is found in primitive hearts such as those of crustaceans,47, 48 in which the muscle cells themselves are branched. Disorientation of myofibrils and cross weaving of myofilaments have been observed in salamander hearts grown in organ culture for 3 months or longer, at which time pronounced changes of cellular dedifferentiation also developed,49 and in rhabdomyomas.50–53

The preceding evidence indicates that disarray of myofibrils occurs in primitive or incompletely differentiated muscle cells and also that this change can develop in cells in which myofibrils were previously arranged normally. These observations are of particular relevance to the problem of IHSS, in which other features, such as increased extent of cellular branching and the presence of large side-to-side intercellular junctions, are also suggestive of a less differentiated cell type than that found normally in adult myocardium.

Functional Significance of Ultrastructural Alterations in IHSS

The abnormal orientation of myofibrils and myofilaments in IHSS may be a result of altered mechanical stresses related to increased cellular branching and abnormal arrangement of cells. As mentioned previously, myofibrils are randomly oriented during the early stages of embryogenesis of cardiac muscle cells. Although little is known of the mechanisms that govern the final orientation of myofibrils during growth of cardiac muscle cells, Manasek46 has suggested that the mechanism of alignment involves groups or bundles of cells rather than isolated cells, and that myofibrils are aligned within specific regions of the heart as a response to stresses induced by contraction. As pointed out by Manasek, contraction induces linear stresses within a cell, and eventually myofibrillar alignment coincides with these forces, but the existence of a causal relation has not been established unequivocally. It is well known, however, that mechanical forces determine the patterns of orientation of other structural fibrous proteins such as those in bone and tendon. If the oblique or transverse orientation of myofibrils of cardiac muscle cells in IHSS were dependent on mechanical forces exerted by adjacent cells, one would expect to find the same myofibrillar orientation in cell-branching areas immediately adjacent to each side of an intercellular junction separating two cells. Figure 2 shows that this was indeed the case. For these reasons we consider that the oblique or transverse arrangement of these myofibrils results from abnormal mechanical forces related to the angles of pull exerted on an individual cell by tightly attached, diversey oriented neighboring cells.
Therefore, it may be concluded from the preceding that myofibrillar orientation is altered when mechanical orienting forces are abnormal, as in IHSS, in cells at marginal zones of infarcts (i.e., at the junction between contractile and noncontractile tissue), and in cultured salamander hearts (in which hemodynamics are severely altered), or not fully established, as in embryonic hearts and in cells of the conduction system. We can only speculate as to whether the extensive side-to-side junctions are the factor responsible for the altered tissue architecture in IHSS or whether these junctions represent a consequence of a more basic abnormality of morphogenesis.

The hypertrophied muscle mass in the ventricular septum in IHSS would appear to be very inefficient in terms of producing an orderly pattern of tension development. The overall resultant force vector generated by a group of diversely oriented cells such as those in the septum would be smaller than that of a group of cells oriented in parallel. The force vectors contributed by individual, diversely oriented cells would not be as additive as they would be if the cells were oriented in parallel. Instead, the magnitude of the overall force vector would be a function of the degrees of divergence of the planes of orientation of individual cells. Similar considerations would apply to the force vectors produced by sarcomeres oriented in different directions within a cell. It would seem also that when some of these cells undergo shortening they may exert tension upon other cells oriented in different directions, so that while some cells shorten others may be held isometrically or even in actual stretch. Such a situation would constitute a powerful stimulus to hypertrophy, as does isometric contraction in inducing skeletal muscle hypertrophy. Moreover, this disorderly arrangement of muscle cells would appear to be mechanically unstable, so that with changes in contractility the resultant force vectors of groups of differently oriented cells could undergo more significant alterations in magnitude and direction than would be the case if these cells were arranged in parallel. Thus, these reasons support the hypothesis that abnormal architecture of muscle cells is the basic cause of IHSS, and that this abnormal architecture provides mechanisms for the maintenance of a continuing stimulus to hypertrophy as well as for changes in the severity of the outflow tract obstruction. Although our observations strongly suggest that this abnormality is congenital, and that its nature is such that hypertrophy will develop with time and various other factors, studies of myocardial ultrastructure in patients with early stages of IHSS are needed to evaluate this possibility. We have not observed significant abnormalities in the arrangement of muscle cells, myofibrils, and myofilaments in a series of 40 biopsies (20 taken from left ventricle with a Menghini needle and 20 obtained from the right side of the ventricular septum with the Konno catheter) from the hypertrophied and dilated hearts of patients with congestive, nonobstructive cardiomyopathies (Unpublished data). Except for previous observations on IHSS we are not aware of any studies of cardiac hypertrophy in which these architectural changes have been described. For these reasons we do not believe that such changes are nonspecific features of advanced degrees of cardiac hypertrophy and failure. Data on the exact distribution of these changes throughout the hearts of patients with IHSS are indispensable to assess the extent to which structural and functional abnormalities correlate in these patients. Additional studies of the patterns of hypertrophy, particularly in the late stages of concentric hypertrophy, will be necessary to determine (1) whether or not these changes occur as the result of extreme cardiac hypertrophy or severe interstitial fibrosis in conditions other than IHSS, and (2) whether or not the mechanisms of myocardial dysfunction postulated for IHSS apply also to advanced stages of cardiac hypertrophy due to other causes.

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Myocardial Ultrastructure in Idiopathic Hypertrophic Subaortic Stenosis: A Study of Operatively Excised Left Ventricular Outflow Tract Muscle in 14 Patients

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