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Ultrastructure of Myocardium in the Hurler Syndrome

Possible Relation to Cardiac Function * **

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Summary. Cardiac tissues obtained at post mortem examination of eight patients with the Hurler syndrome, who ranged in age from 5 to 23 years, were examined by histochemical methods and electron microscopy. Extensive myocardiocytic vacuolization and increased interstitial fibrous tissue were noted by light microscopy in all hearts. The cytoplasmic (perinuclear) vacuoles contained Luxol-fast-blue-positive substance. At the ultrastructural level, abnormal cytoplasmic organelles were present within the myocardiocytes in all patients. These organelles were of three types: zebra bodies (ZB), membranous cytoplasmic bodies (MCB) and granulomembranous bodies (GMB). As ZB and MCB are believed to represent the morphological counterpart of accumulated gangliosides, these substances rather than glycosaminoglycans appear to be stored within myocardiocytes of patients with the Hurler syndrome. The accumulation of gangliosides and the consequent damage to the myocardial substratum probably contributes to the clinically evident cardiac disease, so often observed in the patients with this disorder.

Key words: Hurler syndrome – Myocardium – Histochemistry – Ultrastructure – Storage of gangliosides

Introduction

The Hurler syndrome (Mucopolysaccharidosis type IH, MPS type IH), the prototype of the genetic mucopolysaccharidoses, is characterized by the accumulation of dermatan and heparan sulfates in tissues and their excretion in urine, and a deficiency of alpha-L-iduronidase (Legum et al. 1976; McKusick 1972).

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The disease is associated with corneal opacities, marked skeletal deformities and severe mental retardation, and is inherited as an autosomal recessive trait. The heart is frequently involved. Many patients show clinical evidence of cardiac disease and die in congestive heart failure (McKusick 1972). The degree of failure may not always be explained by changes in the cardiac valves or coronary arteries alone.

To explore the possibility that one of the factors contributing to the cardiac manifestations may be inherent within the myocardium itself, an examination of the hearts of eight children with the Hurler syndrome was carried out by light and electron microscopy.

The purpose of this communication is to report the results of this investigation and to discuss the possible implications of the findings within the broader concept of the pathogenesis of tissue changes in the Hurler syndrome.

Materials and Methods

Cardiac tissues obtained at autopsy in six male and two female patients (age range from 5 to 23 years) served as the basis for this study. Random sections removed from the atrial and ventricular myocardium were fixed in 10% formalin and processed routinely for paraffin embedding. Sections were stained with Hematoxylin-Phloxine-Saffron (HPS), Masson's trichrome, Resorcin fuchsin – Metanil yellow – Nuclear fast red, and Periodic Acid Schiff – Luxol Fast Blue (PAS-LFB) stains. Myocardial tissues obtained at autopsy from two children, aged 7 and 13 years, with no evidence of cardiac disease, served as controls.

For electron microscopy similar tissue from six patients was immediately fixed in 2% glutaraldehyde. In the remaining two instances, formalin-fixed tissue was utilized and re-processed for electron microscopy. All specimens were post-fixed in 1% osmium tetroxide, embedded in Epon-812, and cut on a Reichert ultramicrotome. One-micron-thick sections were stained with alkaline toluidine blue. Thin sections were stained doubly with uranyl acetate and lead citrate and examined with a Philips-300 electron microscope.

Results

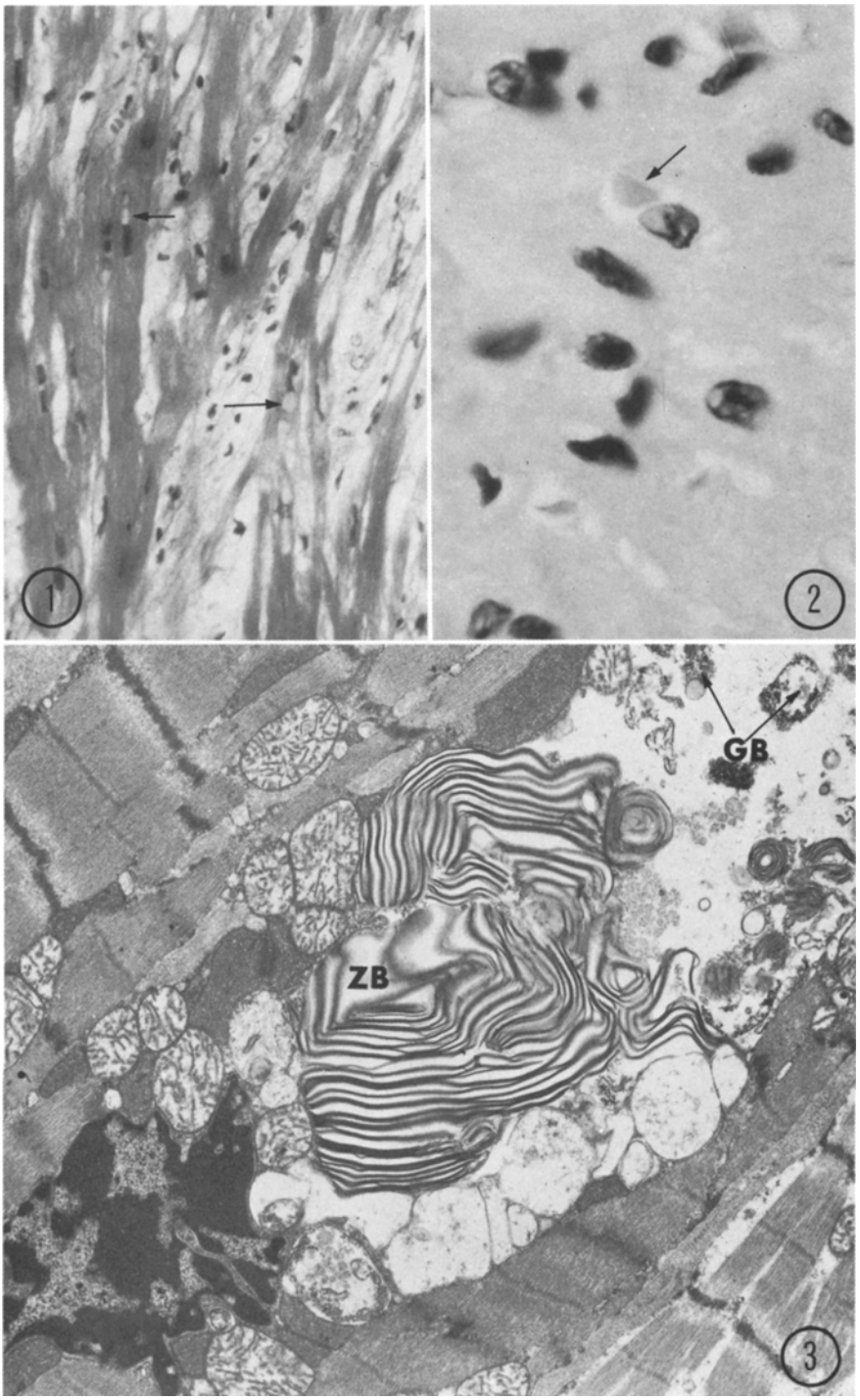
1. Light Microscopy

Interstitial myocardial fibrosis was present in the hearts of all patients (Fig. 1). The fibrous tissue was distributed relatively uniformly throughout the entire thickness of the ventricular wall. The extent of fibrosis varied from one patient to another and did not correlate with the patient's age.

Fig. 1. Left ventricular myocardium from a 10-year-old girl. Note the fragmentation and loss of myocardial fibers and the interstitial fibrosis. Perinuclear vacuolization of myocardiocytes is also seen (*arrows*). Masson's trichrome. ×420

Fig. 2. High power photomicrograph of myocardium from a 6-year-old boy. Luxol-fast-blue positive material is present in the perinuclear vacuole (*arrow*). Periodic acid Schiff-Luxol fast blue. ×920

Fig. 3. Myocardiocyte in the heart of a 23-year-old man contains a large irregular zebra body (*ZB*) composed of stacks of parallel membranes. This organelle is not membrane-bound. Note also the granulo-membranous bodies (*GB*) consisting of irregular electron-dense and more electron-lucent homogeneous bodies. ×8,750


1
2
GB
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3

In addition to the fine fibrosis between individual muscle fibers, there was an increase in the amount of fibrous tissue surrounding the intramural coronary arteries. The perivascular fibrous tissue often extended into the surrounding parenchyma by irregular stellate "processes". This type of fibrosis was more prominent in the inner one-third of the myocardium.

Large confluent patches of fibrous tissue, replacing muscle cells, were noted in only one, i.e., the oldest patient, who was a 23-year-old man.

The myocardiocytes showed prominent vacuolization of the cytoplasm. This change, present in all cases, was evenly distributed throughout the entire width of the atrial and ventricular walls. A correlation between the degree of vacuolization and the extent of interstitial fibrosis was noted. Vacuolization was more prominent in atrial than in ventricular myocardiocytes. In general, only one vacuole, perinuclear in location, was present in each cell. In many myocardiocytes, the vacuole distended the cells and indented their nuclei, thus imparting upon them a "signet-ring" appearance. Some of the vacuoles contained LFB-positive material (Fig. 2). None of the above changes were observed in the control tissues.

2. Electron Microscopy

The most prominent ultrastructural feature concerning the myocardiocytes of all eight patients was the presence of abnormal cytoplasmic organelles. These included zebra bodies (ZB) (Figs. 3–6), membranous cytoplasmic bodies (MCB) (Figs. 7 and 8) and granulo-membranous bodies (GB) (Figs. 3, 6 and 7).

The ZB were present in all eight patients; they were usually irregularly shaped and contained transverse lamellar bands (Fig. 3). The lamellae consisted of alternating light and dark membranes. Fusion of two light membranes resulted in the formation of a dark or thicker membrane. The periodicity of the dark membranes was 5–6 nm. Smaller ZB (approximately 2 by 1.5 μm), found scattered between the myofibrils, were usually membrane-bound (Fig. 4). Larger conglomerations of ZB with other lamellar stacks were often located in the peri-nuclear region and lacked a limiting membrane (Fig. 3).

In six patients the myocardiocytes contained, in addition to the ZB, circular, multi-layered arrays of membranes typical of membranous cytoplasmic bodies (MCB) (Figs. 7 and 8). These measured approximately 1 to 3 μm in diameter. Portions of some MCB were composed of alternating light and dark membranes similar to those observed in the ZB. However, the lamellae of most of the MCB appeared indistinct. Irregular, finely granular material was present at the center of some of the MCB. Although usually less numerous than the ZB, the MCB were very prominent in the tissues of the oldest patient.

The third form of abnormal organelle was of a mixed granulo-membranous nature. These compound configurations, or granulo-membranous bodies (GB), consisted of conglomerates of irregular granules of different size and electron density, lipoid droplets, vacuoles and lamellar arrays (Figs. 3, 6 and 7). The latter were represented by transverse and concentric membranes as well as fragments of lamellae. The GB were not always membrane-bound and they usually occupied a perinuclear area.

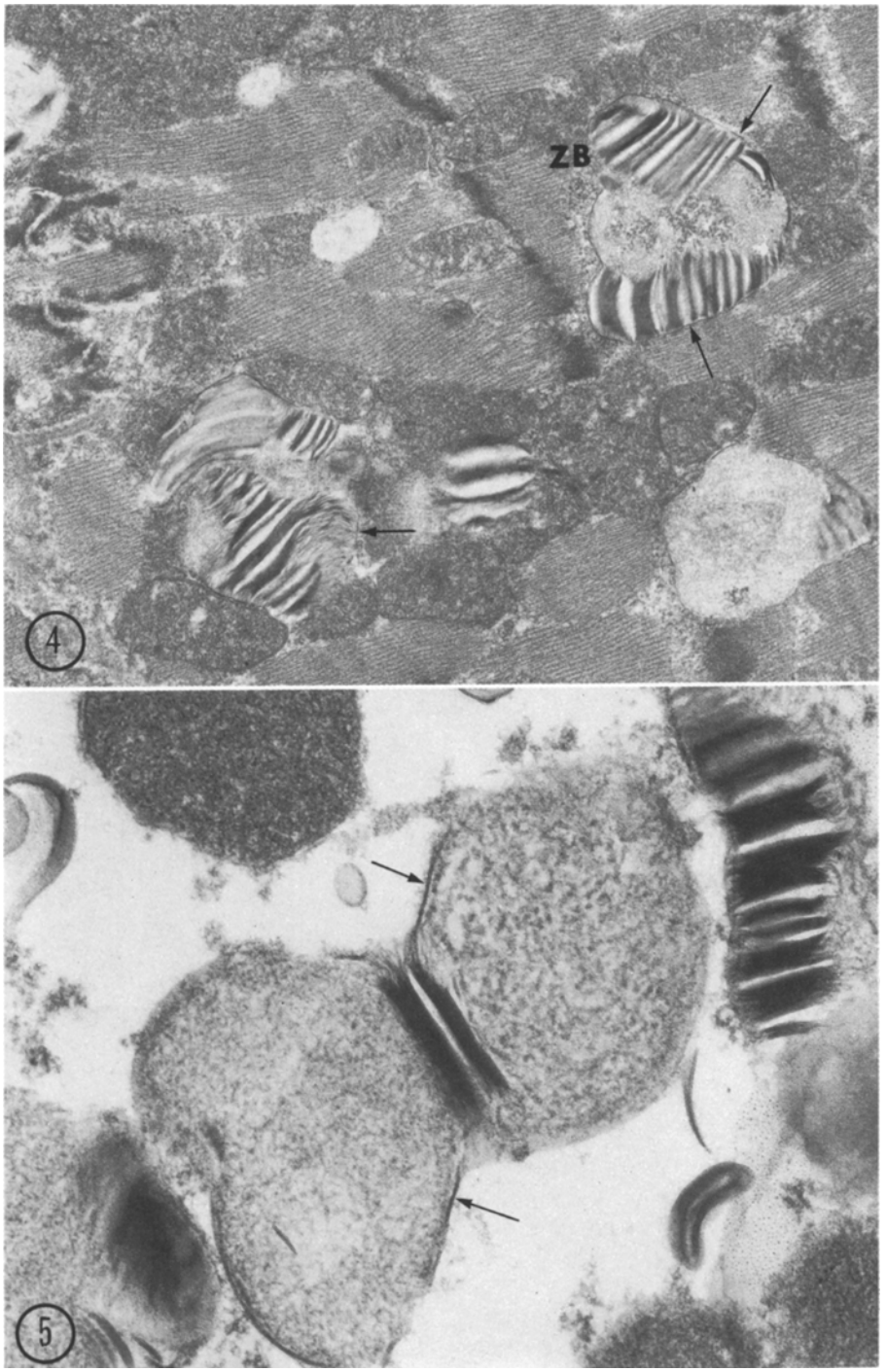

Fig. 4. Myocardiocyte in the heart of 5-year-old boy contains small oval zebra bodies (*ZB*). These appear to have a limiting membrane (*arrows*). × 16,250

Fig. 5. Tissue as illustrated in Fig. 4. Membranous stacks present within organelles are partially lined by a double membrane (*arrows*) and contain a fragmented tubular inner structure suggesting that the organelles are mitochondria. × 49,000

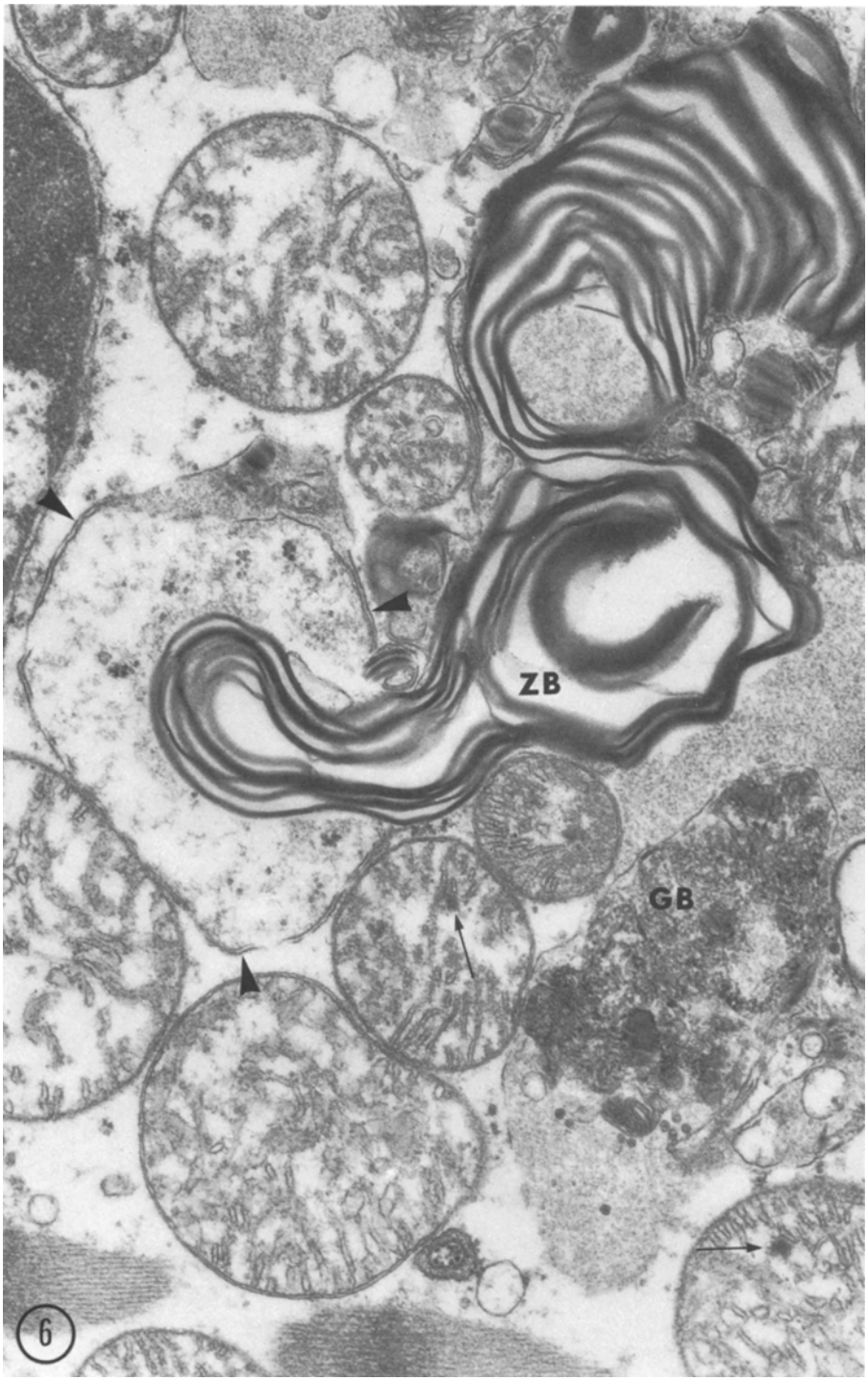

Fig. 6. Tissue as illustrated in Fig. 3. Zebra body (*ZB*) partially contained within a degenerating organelle with a double membrane (*arrowheads*). Some mitochondria contain small irregular electron dense bodies (*arrows*). A granulo-membranous body (*GB*) is also present. ×31,250

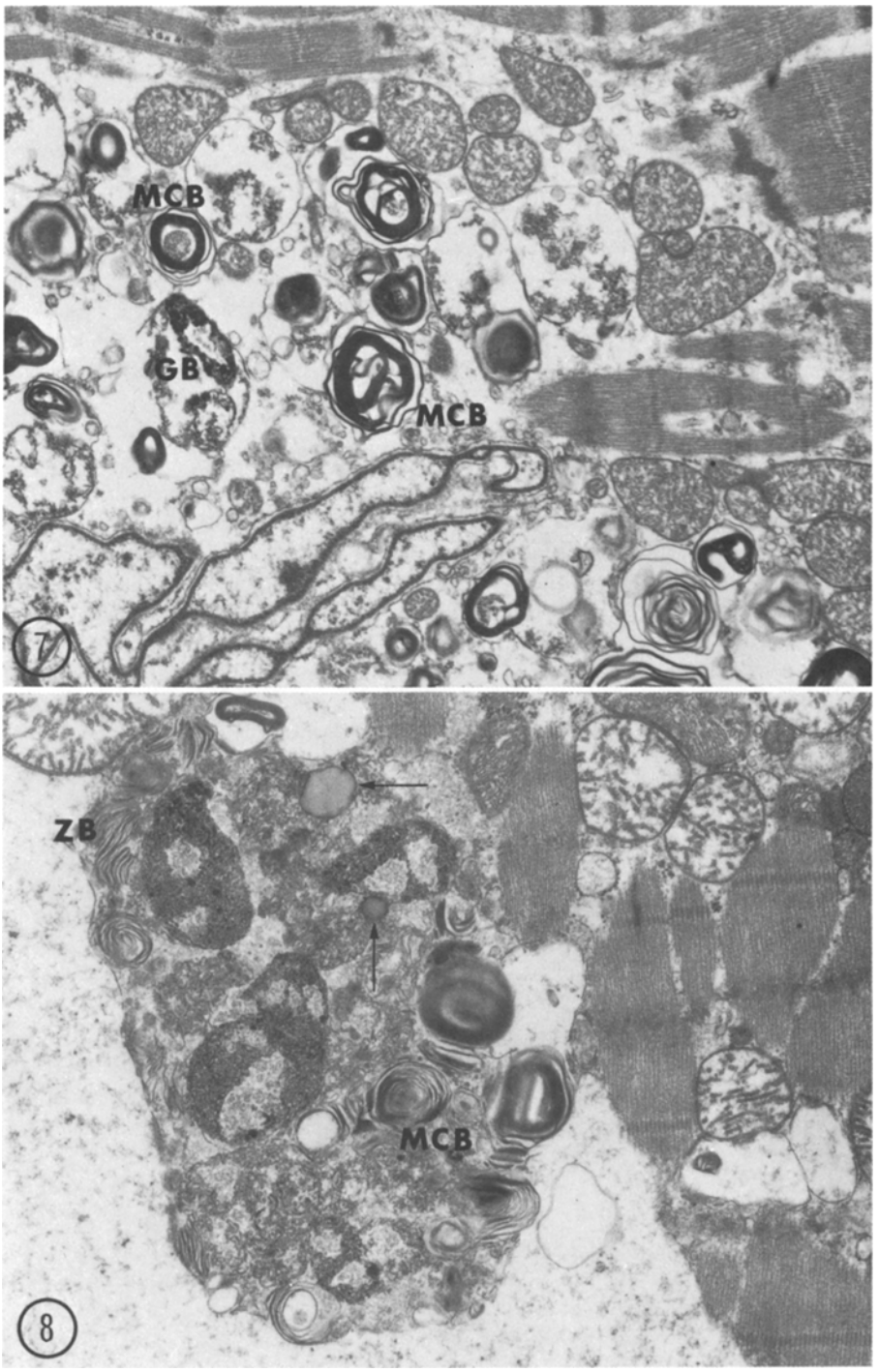

Fig. 7. Tissue as illustrated in Fig. 3. Myocardiocyte with numerous concentric lamellar arrays, i.e., membranous cytoplasmic bodies (*MCB*). Note the disruption of myocardial fibrils. A few granulo-membranous bodies (*GB*) are also present. ×7,250

Fig. 8. Tissue as illustrated in Fig. 3. Abnormal cytoplasmic organelle is seen with membranous arrays of concentric lamellae (*MCB*) and parallel stacks (*ZB*). A few small vacuoles filled with a homogeneous, lightly stained material (suggesting lipid droplets) (*arrows*) are intermingled with other non-descript granular material. The larger electron dense bodies containing areas of rarefaction may represent nuclear substance. ×13,250

Often, the ZB and MCB were related spatially to mitochondria, and in some instances appeared to be developing in close association with or even within degenerating forms of these organelles (Figs. 5 and 6). Many mitochondria contained one or two irregular electron-dense bodies and, occasionally, a linear crystalloid inclusion, and some were of larger than normal size (Fig. 6). Otherwise they appeared normal with respect to number and configuration.

The myofibrils were often disrupted by the presence of the abnormal organelles. The only other abnormality of the myocardiocytic contractile elements was the occasional finding of indistinct or mottled Z-lines. The nuclei, sacroplasmic reticulum, T-tubule system, and intercalated disks were unremarkable. No increase in lipofuchsin inclusions was apparent.

Isolated large cells with numerous cytoplasmic membrane-bound vacuoles were present in the interstitium. These vacuoles contained a varying amount of a granulo-flocculent material resembling that observed in visceral and connective tissue cells elsewhere in the body in this syndrome (Haust 1973; Haust et al. 1969; Lagunoff et al. 1962; Haust and Landing 1961). A second type of abnormal cell noted in the interstitium appeared shrunken and ghost-like, and had many GB in the cytoplasm. It was not always possible to determine whether these represented remnant myocardiocytes or alternatively, tissue macrophages. These and other features of the interstitium were not explored in detail and will be the subject of another study. Similarly, the coronary arteries and their intramyocardial branches were not included in the present study, as they were the subject of a previous investigation (Haust 1971).

Discussion

Clinical evidence of heart disease is found often in patients with the Hurler syndrome. These patients usually die before the age of 10 years of a respiratory infection and/or from cardiac failure (McKusick 1972). In a series of 21 patients with this disease examined by Berenson and Geer (1963), three-fourths manifested heart disease clinically. Lindsay (1950) reviewed 25 reported instances of the Hurler syndrome studied at post-mortem examination. In 17 there was gross or microscopic evidence of cardiac disease, and in 14 of the 19 patients in whom the cause of death was recorded, it was attributed to cardiac failure.

Previous studies have indicated that the major morphologic findings in the hearts of patients with the Hurler syndrome were marked valvular and endocardial thickenings resulting from an increased amount of dense hyalinized collagen and the presence of many large "clear" cells (McKusick 1972). The clear cells ("balloon" or "gargoyle" cells) are believed to be fibroblasts containing cytoplasmic vacuoles filled with stored glycosaminoglycans. Large numbers of clear cells have also been found within the thickened tunica intima of the coronary arteries. Okada et al. (1967) reviewed the changes in the hearts of 40 patients and found that the valves appeared to be the most prominently affected cardiac components. Changes in the mitral valve were seen in 90% of these hearts, in the tricuspid and aortic valves in 40% each, and the pulmonary valve in 20%. In 40%, the coronary arterial lumina were narrowed.

In addition to abnormalities found in the cardiac valves and coronary arteries, changes have been demonstrated in the myocardium. The hearts of five patients, examined by Renteria et al. (1976) contained increased interstitial fibrous tissue in all. The authors postulated that the fibrosis may have resulted from vascular or valvular disease or the presence of clear and granular cells noted within the myocardial interstitium. The granular cells, first described in the heart of a patient with the Hurler syndrome by Lagunoff et al. (1962), were observed only by electron microscopy or toluidine blue stained sections of plastic embedded material. In contradistinction to the clear cells, the granular cells probably do not contain stored glycosaminoglycans, but rather glycolipids. It was postulated that they are fibroblasts also capable of producing collagen (Renteria and Ferrans, 1976).

Alterations reported to occur in the myocardiocytes themselves include vacuolization of the cytoplasm (Okada et al. 1967), and the presence of abnormal cytoplasmic organelles, i.e., ZB (Renteria et al. 1976). The latter were assumed to represent accumulated "glycolipids" (Renteria et al. 1976).

The ZB, first described by Olszewski within the neurones of patients with the Hurler syndrome (Aleu et al. 1965), have also been found within several other tissues in this and similar syndromes. For example, in the Hurler syndrome, ZB have been reported in neurones (Aleu et al. 1965; Haust 1973; Lach and Haust 1975a), choroid plexus (Lach and Haust 1972), myocardium (Renteria et al. 1976), hepatic Kupffer cells (Haust et al. 1969), Merkel and Schwann cells of the skin (Belcher 1972) and beta-1 basophils of the pituitary gland (Schochet et al. 1974). The ZB have been observed also within neurones (Escourolle et al. 1966; Wallace et al. 1966; Lach and Haust 1975b) and neuroglia (Lach and Haust 1975a) in the Sanfilippo syndrome, as well as neurones of GM_2-gangliosidoses (Adachi et al. 1969) including the Sandhoff disease (Resibois et al. 1970). Lastly they have been noted in myocardiocytes of patients with Sandhoff disease (Blieden et al. 1974) and Fabry disease (Ferrans et al. 1969). When found within neurones, ZB were believed to represent the morphological equivalent of stored gangliosides (GM_2 and GM_3 in the Hurler syndrome) (Aleu et al. 1965).

The MCB differ from ZB by being composed of concentrically arranged lamellae with alternating light and dark membranes. Terry and Korey (1960) described these organelles first within the neurones of a patient with GM_2-gangliosidosis. Analysis of MCB isolated from the neurones of patients with that disease indicated that they contain 35–50% (dry weight) gangliosides as well as much cholesterol (Samuels et al. 1963).

In the present study, cytoplasmic vacuolization of the myocardiocytes was a prominent finding in all cases examined by light microscopy and the degree of vacuolization correlated well with the severity of interstitial fibrosis. When present, the substance in the "vacuoles" gave a positive reaction with Luxol fast blue, a stain widely used for the demonstration of lipoid substances including various gangliosides. The vacuoles appeared to correspond to the large perinuclear "pools" of abnormal organelles noted on electron microscopy. Thus, the histochemical and ultrastructural observations in this study (i.e., the presence of ZB and MCB) suggest that the stored material consists largely of gangliosides.

If this interpretation is correct, then some conceptual aspects of the pathogenesis of the Hurler disease, and perhaps also of other genetic mucopolysaccharidoses, must be re-evaluated. It has been held widely that, with the exception of the nervous system, the injury to all other tissues in the Hurler syndrome results from the accumulation of glycosaminoglycans (Haust 1973; Pennock and Barnes 1976), whereas storage of gangliosides is responsible for the impairment of the former. In fact, cells filled with glycosaminoglycans are present in the connective tissue of the heart, but the morphological equivalents of gangliosides are found within the myocardiocytes. Does this indicate that the metabolic pathways of myocardial cells either resemble those of neurones or that there is an as yet unknown common pathway ultimately responsible for both the accumulation of gangliosides and glycosaminoglycans?

It is likely that the continuous accumulation of storage material in myocardiocytes results in cellular necrosis with consequent interstitial fibrosis. Moreover, the contractile function may be abnormal in those myocardiocytes in which the "metabolic injury" is not lethal. Myocardiocytic dysfunction may contribute to the cardiac failure common in the patients with the Hurler syndrome.

The Hurler syndrome is believed to represent one of the lysosomal storage diseases as there is conclusive evidence that a deficiency of the lysosomal enzyme, alpha-L-iduronidase, results in a defective catabolism and accumulation of glycosaminoglycans (Sly 1980). The present study, however, shows conclusively that gangliosides are also being stored within the myocardiocytes and possibly contribute to the clinically manifest disease. Whereas the mechanism of the accumulation of gangliosides in myocardiocytes has not been established, the ultrastructural observations in the present study suggest some mitochondrial involvement (Figs. 5 and 6).

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