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Coronary Intimal Sclerosis in Morquio's Syndrome

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Summary. Mitral valve, coronary arteries, cartilage, and liver were studied by light and electron microscopy in a 15 year old boy with Morquio's syndrome, a genetic mucopolysaccharidosis, in which a deficiency of lysosomal hexosamine sulfatase is associated with accumulations of keratan sulfate in various organs. Coronary artery intimal sclerosis was a prominent feature of this disorder. Ultrastructural examination revealed numerous intimal smooth muscle cells containing storage vacuoles consistent with lysosomes. This was associated with marked interstitial deposition of collagen, elastin, and basement membrane material. Recent studies of human and experimental atherosclerosis have demonstrated the accumulation of cholesterol within vascular smooth muscle cell lysosomes. Intralysosomal accumulation of substrates other than cholesterol is also associated with vascular intimal sclerosis in genetic lysosomal disorders such as Fabry's disease and Hurler's syndrome. Lysosomal storage of undegraded substrate may be an important pathogenetic mechanism in the development of sclerotic vascular lesions.

Key words: Lysosome – Atherosclerosis – Morquio's syndrome – Storage disease.

Introduction

Accumulation of cholesterol within lysosomes of vascular smooth muscle cells is a feature of both experimental (Goldfischer et al., 1975; Peters et al., 1974; Shio et al., 1974; Wolinsky et al., 1975) and human (Coltoff-Schiller et al., 1976; Factor et al., 1977) atherosclerosis. This may occur because an excess of circulating substrate is presented to the lysosomes (Goldstein et al., 1975) or, there is a deficiency of lysosomal lipolytic activity (Peters et al., 1973). Examples of the former are laboratory animals fed high-cholesterol diets (Goldfischer et al., 1975; Peters et al., 1974; Shio et al., 1974; Wolinsky et al., 1975) and

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possibly hyperlipidemic humans, while the latter is seen in cholesterol deficiency (Sloan et al., 1972) and Wolman's acid esterase deficiency (Patrick et al., 1973). These observations suggest that atherosclerosis may have features analogous to heritable lysosomal storage diseases.

Among the group of mucopolysaccharidoses in which Morquio's syndrome is classified, Hurler's disease, a genetic condition in which the lysosomal enzyme L-iduronidase is deficient (Wolinsky, 1976; Dorfman et al., 1972), is the only one previously reported to be associated with significant intimal sclerosis. Occlusive coronary artery lesions and accumulation of mucopolysaccharide within arterial smooth muscle cell lysosomes are present in young children with this disease (Goldfischer et al., 1975). Sclerotic coronary artery disease also occurs in Fabry's disease (angiokeratoma corporis diffusum) (Desnick et al., 1976), a lysosomal storage disorder characterized by deficiency of α-galactosidase A and accumulation of trihexosyl and digalactosyl ceramides (Sweeley et al., 1972; Desnick et al., 1973).

The present report provides additional evidence that the accululation of metabolites other than cholesterol within lysosomes is associated with accelerated development of intimal sclerosis. A case of Morquio's syndrome (mucopolysaccharidosis type IV with hexosamine 6-sulfatase deficiency (Dorfman et al., 1976)) with typical biochemical and skeletal abnormalities was studied by light and electron microscopy. Coronary intimal sclerosis, which has not been described in this disease, was prominent. Ultrastructural study of the vessel wall revealed the presence of lysosomal vacuoles distended by non-lipid material.

Case Report

The patient was a 15 year old Puerto Rican male admitted to the hospital because of difficulty in breathing. He was the product of a non-consanguinous marriage, with one full sib and two half-sibs unaffected. Development was normal during the first year of life, but progressive motor deficits and skeletal abnormalities were observed thereafter until age 6, when the diagnosis of Morquio's syndrome was entertained on clinical grounds. Definitive diagnosis was made two years later when biochemical analysis of a 24 h urine sample revealed 112 mg/l keratosulfate. Subsequently, the patient became bedridden and progressively deformed. He was admitted to the Bronx Municipal Hospital Center on multiple occasions for respiratory insufficiency and infections.

On his final admission, the patient was noted to be blind and quadriplegic, with respiratory distress, dehydration, and fever. He was treated with intravenous fluids, antibiotics, and intubation. Over several days his respiratory status deteriorated and he died 5 days after admission.

Autopsy was performed 12 h after death. Typical corneal clouding and skeletal abnormalities of Morquio's syndrome were apparent. The gross appearance of the internal organs was generally unremarkable, with the exception of the slightly hypertrophied heart which revealed thickened mitral valve leaflets. The aorta and coronary arteries showed focal intimal sclerosis, but the coronary lumens were patent. The lungs were congested without pneumonitis. Compression of the spinal cord and nerve roots by bony abnormalities led to respiratory paralysis, and was the immediate cause of death.

Materials and Methods

Portions of liver and brain tissue were immediately frozen for biochemical analysis. Small pieces of liver, kidney, brain, and cartilage were fixed in 3 per cent buffered glutaraldehyde for 6 h,

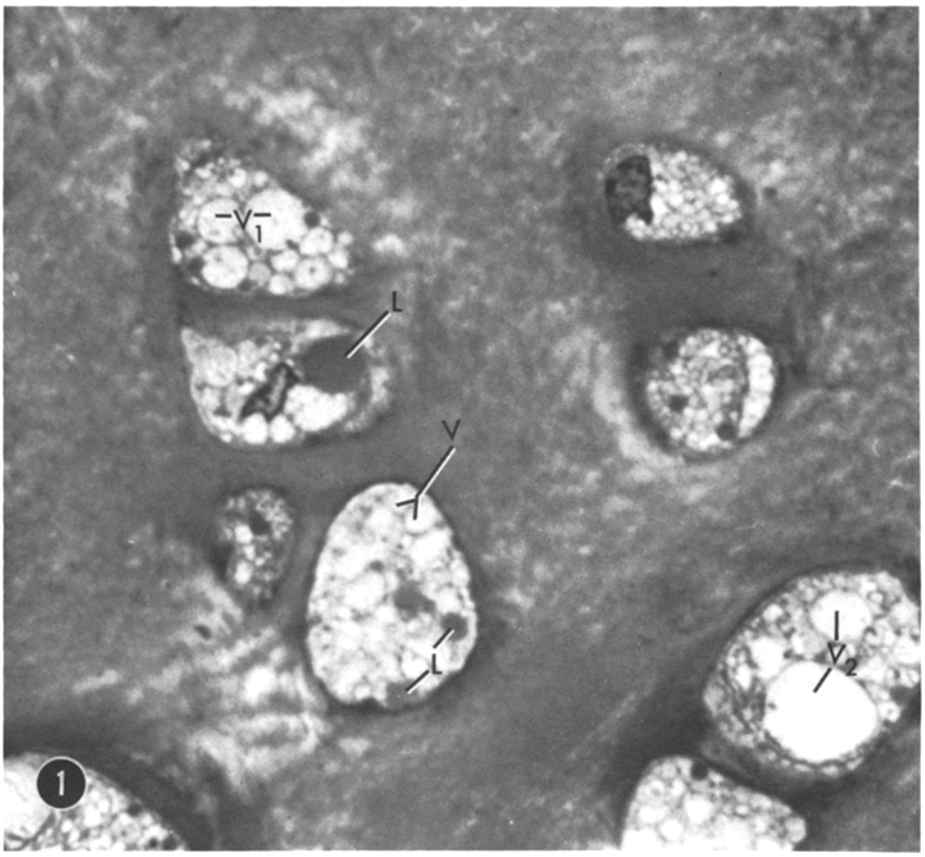

Fig. 1. This one micron section of Epon embedded vertebral cartilage demonstrates that all chondrocytes are abnormally vacuolated. Densely stained osmiophilic lipid (*L*) is contrasted with the more numerous large and small clear vacuoles (*V*) which fill the cytoplasm. Vacuoles V_1 and V_2 contain particulate material which does not stain as intensely as lipid. (Alkaline toluidine blue, ×1150)

and then transferred to cacodylate-sucrose buffer. The heart and aorta were fixed at autopsy in 3.7 per cent buffered formaldehyde. After 4 days of fixation 1 mm cubes of sclerotic coronary artery and aorta, and thickened mitral valve were removed and washed in cacodylate-sucrose buffer. Both the glutaraldehyde and formaldehyde fixed tissues were post-fixed for 2 h in osmium tetroxide, dehydrated in progressively increasing concentrations of alcohol and propylene oxide, and embedded in epoxy resin. One micron sections were prepared and stained with toluidine blue. Appropriate areas were selected, and thin sections were obtained and stained with uranyl acetate and lead citrate. Grids were examined in RCA EMU 3C and Siemens 1A electron microscopes. Paraffin sections for light microscopy were routinely stained with hematoxylin-eosin,periodic acid-Schiff, alcian blue, and van Gieson's elastica stain.

Results

Biochemical analysis of the frozen liver sample performed by Dr. Allen L. Horwitz of the University of Chicago, revealed levels of hexosamine-6-sulfate

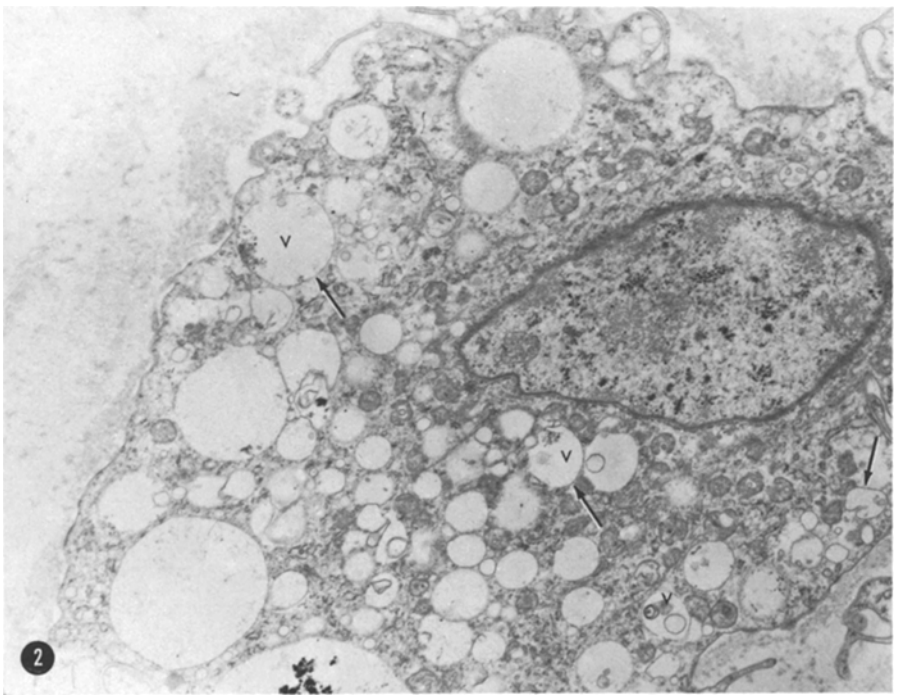

Fig. 2. Multiple vacuoles (*V*) are observed in this cell present within an abnormally thickened and sclerotic mitral valve leaflet. Although many vacuoles appear completely empty, others contain membranous structures, electron dense granules, and flocculent material. The single membrane delimiting the vacuoles is clearly identified (arrows). (×8,400)

sulfatase which were less than 8 per cent of normal, when normalized to other sulfatases. The results of the liver enzyme studies were considered to be consistent with the diagnosis of Morquio's disease (Dorfman et al., 1976).

Histologic study of costal cartilage demonstrated the presence of large abnormally vacuolated chondrocytes. The nature of the vacuolization was seen best in the one micron Epon sections in which the cells were observed to be distended by numerous small and large vacuoles. Two types of vacuoles were present, one containing osmiophilic lipid and the other clear, or with finely granular material (Fig. 1). The two types of vacuoles were also present in the liver, often in the same hepatocyte, while only clear vacuoles were seen in the mitral valve, aorta, and coronary artery. Soluble mucopolysaccharides were presumably extracted by the aqueous fixatives utilized, and neither metachromasia nor PAS and alcian blue reactivity could be demonstrated in the vacuoles.

Ultrastructure of the cartilage and mitral valve demonstrated that the vacuoles were membrane delimited and predominantly empty, but they occasionally contained membranous and electron-dense material. This was most apparent in the mitral valve (Fig. 2), in which some cells were completely filled with these vacuoles. In the liver, hepatocytes had vacuoles with lipid, and others contained a granular flocculent material. Some of these latter vacuoles were

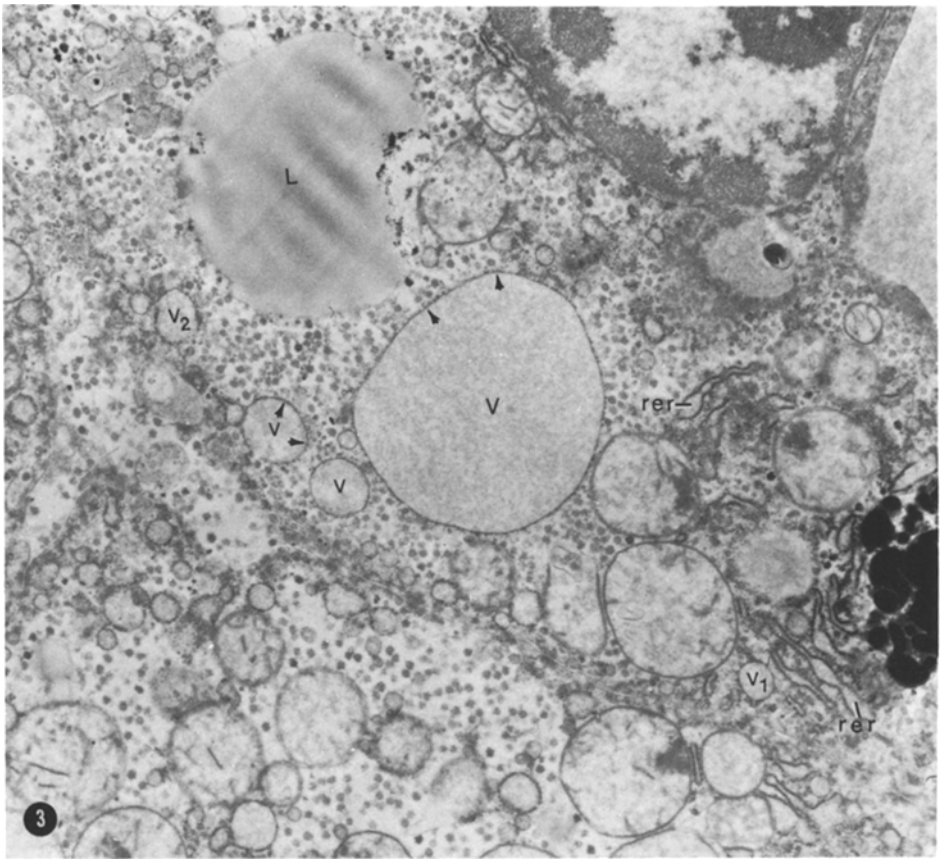

Fig. 3. This section of liver reveals an hepatocyte with characteristic lipid (*L*), and multiple membrane enclosed vacuoles (*V*) containing flocculent material. Cisternae of rough endoplasmic reticulum (*rer*) have saccular dilatations filled with material similar to that noted in the large vacuoles. Vacuoles V_1 and V_2 appear to arise from these dilatations. Many vacuoles have ribosomes (arrowheads) aligned along the outer surface of their delimiting membrane. (× 13,000)

continuous with dilated cisternae of endoplasmic reticulum, while others had ribosomes on their outer surface (Fig. 3).

Most unexpectedly in this young patient, the coronary artery revealed a striking degree of intimal sclerosis (Fig. 4). Similar intimal lesions were observed focally in the aorta. In both tissues, vacuolated intimal cells could be appreciated in the paraffin and Epon-embedded sections. Osmiophilic lipid could not be demonstrated in the vessel wall and the vacuoles were presumed to have contained mucopolysaccharides. Ultrastructural study of the coronary artery demonstrated the presence of identifiable intimal smooth muscle cells filled with empty appearing vacuoles (Fig. 5). Only rare intracellular lipid was seen. The storage vacuoles were delimited by a single unit membrane (Fig. 6), and were therefore considered to be lysosomes. They could be distinguished from swollen degenerated, or poorly preserved mitochondria. The interstitium around the vacuo-

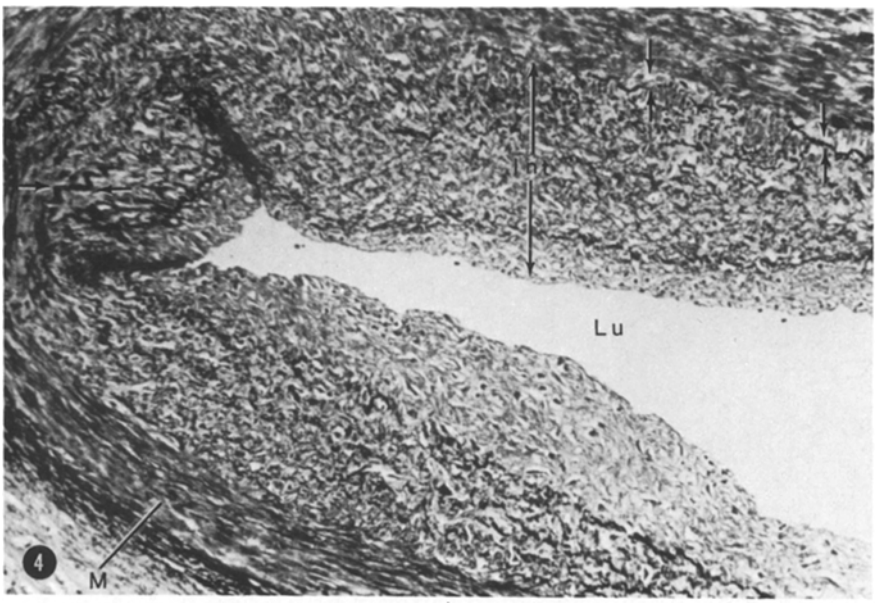

Fig. 4. This representative section of left anterior descending coronary artery demonstrates marked intimal thickening. The arterial lumen (*Lu*) is compromised by an intima (*Int*) which is 2–3 times the thickness of the unremarkable media (*M*). The intima is composed of collagen, multiple irregular fine and coarse elastic fibers, and vacuolated cells. Portions of the internal elastic lamina (small arrows) are noted above the media, however it is discontinuous and fragmented (van Gieson elastic stain, × 220)

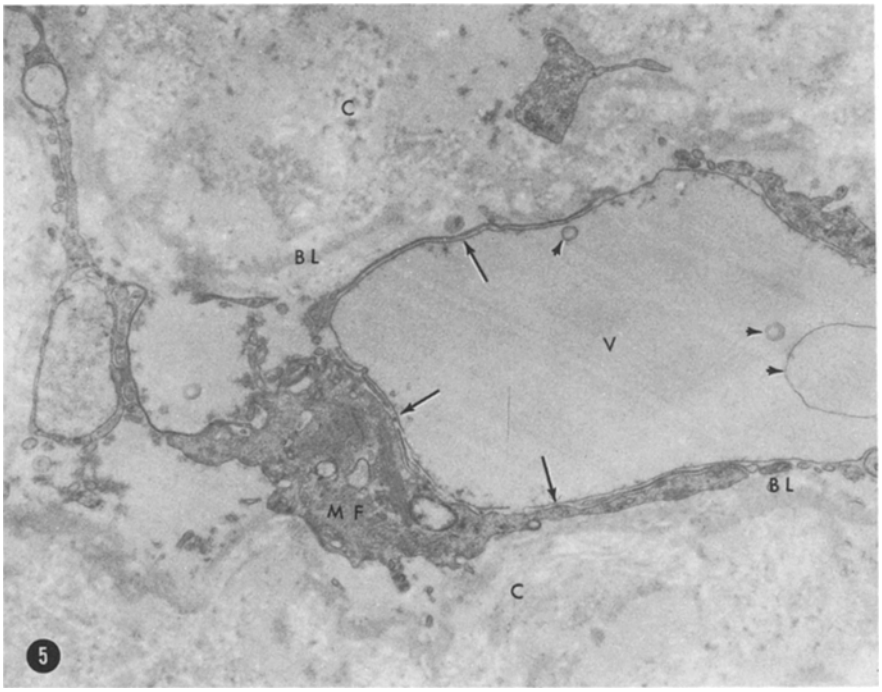

Fig. 5. A representative area of the sclerotic coronary artery intima reveals a smooth muscle cell containing cytoplasmic myofilaments (*MF*), distended by a huge membrane delimited vacuole (*V*). The vacuole is almost entirely empty with the exception of small amounts of flocculent material and several membranous structures (arrowheads). The single membrane (arrows) enclosing the vacuole is focally broken. The interstitium is composed of thickened and reduplicated basal lamina (*BL*), and numerous collagen fibers (*C*) (× 13,000)

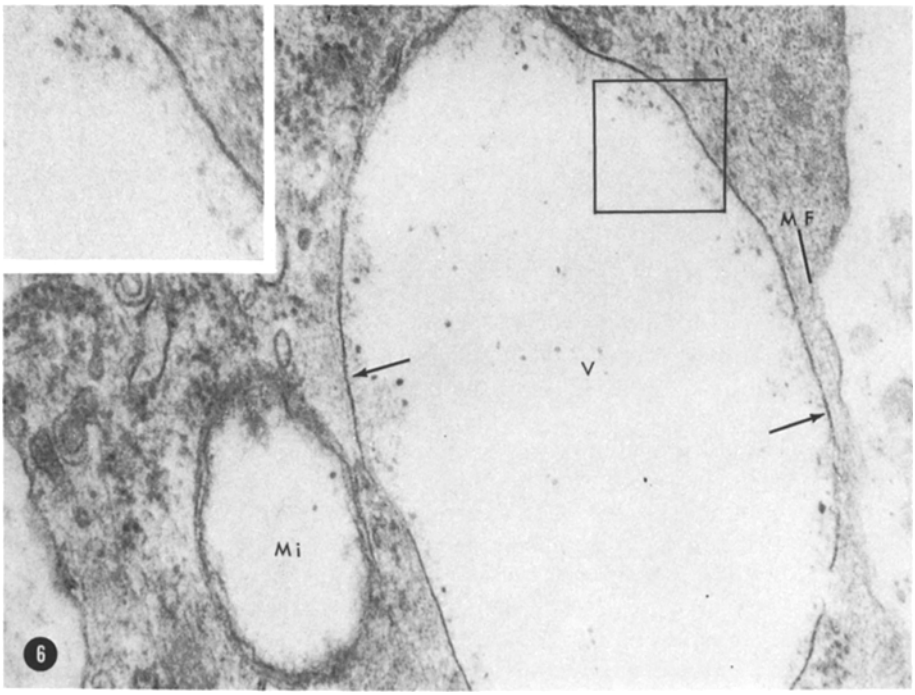

Fig. 6. This smooth muscle cell from the coronary artery intima is identified by abundant cytoplasmic myofilaments (*MF*). A large vacuole (*V*) contains residual flocculent and granular material. The vacuole is delimited by a single unit membrane (arrows), in contrast to the double membrane which surrounds the swollen, degenerated mitochondrion (*Mi*) next to it. The fact that the vacuole is enclosed by a single membrane supports the view that this structure is of lysosomal origin. The trilaminar arrangement of the unit membrane can be appreciated in the inset which represents a high magnification view of the area within the square. ($\times 70{,}000$, inset $\times 132{,}000$)

lated smooth muscle cells contained increased amounts of collagen, elastic tissue, and basement membrane-like material (Fig. 5).

Discussion

Morquio's syndrome, among the group of hereditary mucopolysaccharidoses, is unique in that it is the only one in which keratosulfate is excreted in large amounts in the urine (Van Hoof, 1973). This substance which is most prominent in cartilage and cornea, accounts for the skeletal deformities and ocular opacities characteristic of this disease (McKusick, 1972). Despite the fact that Morquio's syndrome is a systemic metabolic disease which affects diverse organs (McKusick, 1972), little attention has been paid to abnormalities of the cardiovascular system. Although it is generally appreciated that cardiac valves may be affected (Schieken et al., 1975), only one brief description of intimal vascular sclerosis could be found (Schenk et al., 1974).

Previous ultrastructural studies of Morquio's syndrome have demonstrated the presence of swollen lysosomes which are either empty or which contain membranous or granular material, in brain (Gilles et al., 1971), skin (Hollister et al., 1975), cartilage (Hollister et al., 1975; Maynard et al., 1973), and liver (Tondeur et al., 1969). These distended lysosomes are similar to those observed in the other genetic mucopolysaccharidoses (Belcher, 1972; Callahan et al., 1966; De Cloux et al., 1969; Goldfischer et al., 1975; Lagunoff et al., 1972; Lagunoff et al., 1966; Van Hoof, 1973; Wallace et al., 1966) in which the stored substances are dermatan sulfate and heparan sulfate (Van Hoof, 1973). The fact that these two mucopolysaccharides are normal constituents of blood vessels (Neufeld, 1972), may account for the severity of occlusive vascular disease in young children with Hurler's disease (Goldfischer et al., 1975). Our observations indicate that Morquio's syndrome also must be included in the group of lysosomal storage diseases associated with vascular sclerosis.

Intensive study has focused on lysosomal involvement in atherosclerosis, particularly with the mechanisms of lipid uptake, internalization within the vascular smooth muscle cell, and digestion by lysosomal cholesteryl esterase (Wolinsky, 1976). Intralysosomal lipid has been demonstrated in both early and advanced stages of atherosclerosis (Coltoff-Schiller et al., 1976; Factor et al., 1977), and has even been observed in aortic smooth muscle cells of the neonate (unpublished observations: 1977). The relationship between lysosomal storage of cholesterol and the connective tissue and cellular proliferation that characterizes intimal sclerosis is not understood. The presence of intimal sclerosis in Morquio's syndrome and Hurler's disease, two disorders in which lipid storage is not significant, suggests that the overloading of the lysosomal digestive apparatus may be a central pathogenetic event in the development of occlusive vascular disease.

To focus on the role of lysosomal storage of substrate in the evolution of intimal vascular lesions, we have proposed a new term: thesaurosclerosis. Hurler's disease (Goldfischer et al., 1975), Fabry's disease (Desnick et al., 1976), and now Morquio's syndrome are three inherited disorders in which lysosomal storage of metabolites other than cholesterol, appears related to the development of intimal sclerosis. Another lysosomal storage disorder may be considered in the same category. Pompe's disease (glycogenosis type II) commonly results in endocardial fibroelastosis and myocardial, hepatic and muscular glycogen accumulation (Baudhuin et al., 1964; Hernandez et al., 1966). One report also exists in which intramyocardial vessels were stenosed by swollen endothelial cells containing lysosomal glycogen (Garancis, 1968). True vascular sclerosis may not develop in these children because they typically die young, usually before one year of age.

As awareness of lysosomal mechanisms in vascular disease increases, other thesaurosclerotic disorders may be described with different substrates. Alkaptonuria has been associated with severe atherosclerosis and cardiac valvular lesions typified by fibrosis, calcification, and staining with ochronotic pigment (Lichtenstein et al., 1954). As with Morquio's syndrome, the major manifestations are in the skeleton and joints, and most morphologic studies have focused on these areas. Studies of the vessel wall have not specifically localized the pigment (Fuch, 1968), but it is likely that it will be demonstrated within smooth muscle

cell lysosomes, since it has already been observed in membrane bounded intracytoplasmic organelles in synovial cells and macrophages (Kutty et al., 1973).

Although the relationship between the accumulation of undigested material within lysosomes and the proliferative and synthetic lesions of intimal sclerosis is unknown, it is likely that the lysosome, the cellular digestive apparatus, must play an essential role in this process. The situation in the vessel wall may be analogous to what occurs in silicosis and asbestosis. In both of these conditions the stored material is exogenous in origin. Neither silica, probably because of its toxic effect on the lysosomal membrane (Allison et al., 1966; Nadler et al., 1970), nor asbestos particles, because of their size (Suzuki et al., 1969), can be effectively sequestered within lysosomes. Both situations result in chronic sclerotic lesions in the pulmonary interstitium or pleura.

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