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Infantile acid maltase deficiency

I. Muscle fiber destruction after lysosomal rupture

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Summary. The loss of normal ultrastructure of skeletal muscle during the relentless course of infantile acid maltase deficiency (AMD) is re-examined in the light of the lysosomal rupture hypothesis. This hypothesis suggests that movement and increased myofibril rigidity during contraction cause lysosomes in muscle to rupture and release glycogen and other lysosomal contents to a much greater extent than do lysosomes in other cell types in cases of infantile AMD. Muscle fibers are destroyed, while macrophages and other cells mostly accumulate glycogen in storage lysosomes without being destroyed. When morphological stages of fiber destruction are placed in a sequential series, from fibers most like normal infant muscle to those with only remnants of muscle ultrastructure, the earliest stages seen contain intact storage lysosomes. Intermediate stages exhibit ruptured lysosomal membranes and free glycogen as well as glycogen in lysosomes. Loss of myofibrillar material and loss of glycogen occur in later stages of fiber destruction.

Membrane-enclosed glycogen and mitochondria are relatively protected from the process of destruction. The electron-microscopic observations support the lysosomal rupture hypothesis and are compatible with the original proposal of Hers, that the disease results from a deficiency of a single lysosomal enzyme. Secondary changes other than muscle fiber destruction probably relate to disrupted control mechanisms and the nature of muscle as a specialized cell. At least two different mechanisms could contribute to the loss of contractile activity and myofibrillar structure.

Key words: Glycogenesis II – α-glucosidase deficiency – Lysosomal deficiency disease – Pompe's disease

Introduction

Infantile acid maltase deficiency (Pompe's disease) is a severe, progressive disease, relentless in its course and resulting in death within two years of

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birth, usually within the first year. Muscle, both skeletal and cardiac, is particularly affected, and death usually results from respiratory or cardiac insufficiency. The modern concepts of Pompe's disease originated with Hers (1963, 1965), who identified the specific lysosomal enzyme deficiency.

The defect, in which glycogen granules accumulate within lysosomal membranes, is generalized in the infantile form, as lysosomes throughout the body lack acid maltase (α-glucosidase), which seems to be the only enzyme that removes glycogen from lysosomes. Membrane-enclosed glycogen has been detected in Pompe's disease in many different organs and cell types (Baudhuin et al. 1964; Cardiff 1966; Garancis 1968; Martin et al. 1973) even in utero (Hug et al. 1973).

Reviews (Bosch and Munsat 1979; Engel 1973; Hers 1973; Hers and de Barsy 1973; Hug 1972; Schotland 1973) and recent studies (Baudhuin et al. 1964; Cardiff 1966; Engel 1970; Garancis 1968; Hudgson and Fulthorpe 1975; Hug et al. 1966; Hug and Schubert 1967; Martin et al. 1973; Sarnat et al. 1982; Schnabel 1971; Wolfe and Cohen 1968; Zellweger et al. 1955) have focused on three areas of difficulty in applying the lysosomal (single enzyme) deficiency disease concept of Hers (1963, 1965) to infantile acid maltase deficiency (AMD): 1) the severe effects on muscle, as compared to other tissues, 2) the large amounts of free glycogen present in muscle in infantile AMD, and 3) the presence in muscle of basophilic (metachromatic) material with staining properties different from glycogen.

This study focuses primarily on the first of these topics, the striking muscle fiber destruction that characterizes infantile AMD. A specific hypothesis is proposed, that the inner environment of muscle fibers contributes to physical rupture of the membrane barrier that normally separates lysosomal contents from the cytoplasm. To evaluate this hypothesis, labeled the "lysosomal rupture hypothesis", muscle fibers affected by infantile AMD are studied with the electron microscope. Attention is focused on the membrane barrier between lysosomal contents and cytoplasm in muscle fibers at different stages of the continuum of fiber destruction.

Ten years after his description of AMD as the first recognized lysosomal deficiency disease (1963), Hers (1973) reviewed the many additional lysosomal diseases and stated: "Rupture of the lysosomal membrane secondary to its distention is also a possible event, which, however, has no yet been demonstrated in inborn lysosomal diseases; it seems to occur in acute gout (Weissmann 1971) and in silicosis (Allison 1971)." The present manuscript presents evidence of lysosomal rupture in infantile AMD and suggests that results following from this rupture can reasonably account for the observed destruction of muscle fibers.

Materials and methods

Electron microscopy was performed on human skeletal muscle biopsy specimens submitted to the Division of Neuromuscular Pathology. The illustrations herein show deltoid muscle from a 5-month-old patient with infantile acid maltase deficiency, who died less than 3 months later. The muscle was fixed with 2.5% glutaraldehyde in Millonig's phosphate buffer, pH 7.4, post-osmicated, and embedded in Epon 812. Biopsies from four patients with infantile AMD,

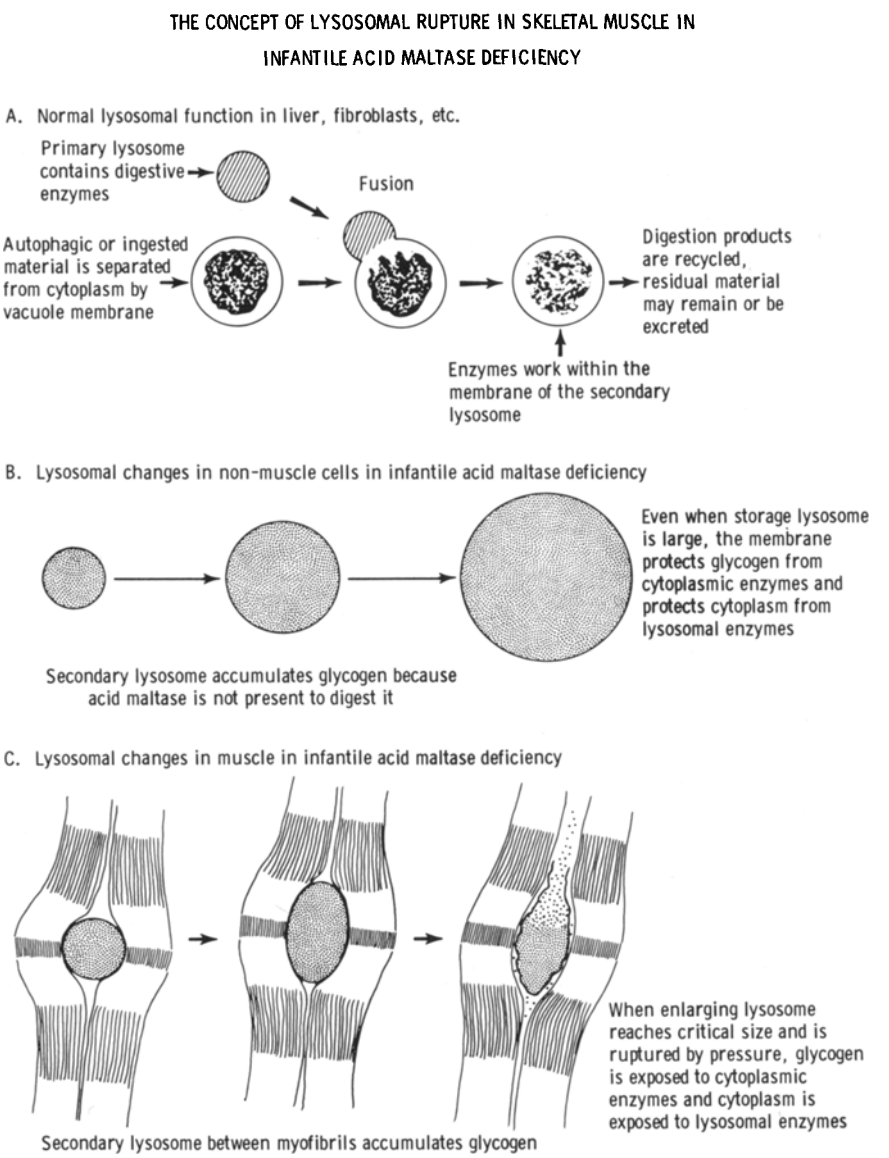


Fig. 1. Diagram of lysosomal rupture hypothesis

originally fixed in buffered formalin and recovered from paraffin, post-osmicated, and embedded in epoxy resin, were not as well preserved. Thin sections were usually stained with uranyl acetate and lead citrate. We followed the description of Anderson (1972) of the methods of Thiery (1967) for periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP) and periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP).

The lysosomal rupture hypothesis

A diagram of the proposed mechanism of rupture is presented in Fig. 1. Muscle differs from other cells in that the abnormal storage lysosomes form between myofibrils that move and

increase in rigidity during normal contraction and leave little space for abnormal inclusions. The physical rupture of lysosomal membranes is postulated to occur when the increased size of the glycogen vacuoles within limited space produces sufficient stress.

Results

The initial light- and electron-microscopic observations showed much variation between muscle fibers in infantile AMD. As an aid in interpreting the process of fiber destruction, the fibers in micrographs are placed in a sequential series, from fibers most like normal infant muscle to those with the most extensive destructive changes. The following list presents sequential stages arbitrarily imposed upon what seems a continuum. The staging of the fibers chosen as illustrations can be correlated with this list.

Stage A. Muscle fibers are normal in appearance except for membrane-enclosed glycogen between myofibrils. Glycogen aggregates have sharp outlines. Glycogen free between myofibrils is sparse.

Stage B. This stage can be distinguished from stage A only with the electron microscope. The glycogen aggregates between myofibrils exhibit broken membranes. Excess glycogen is free in the sarcoplasm.

Stage C. Myofibrils remain mostly connected and aligned. Glycogen may separate myofibrils or occupy glycogen lakes and fill up to half of the cross sectional area of fibers.

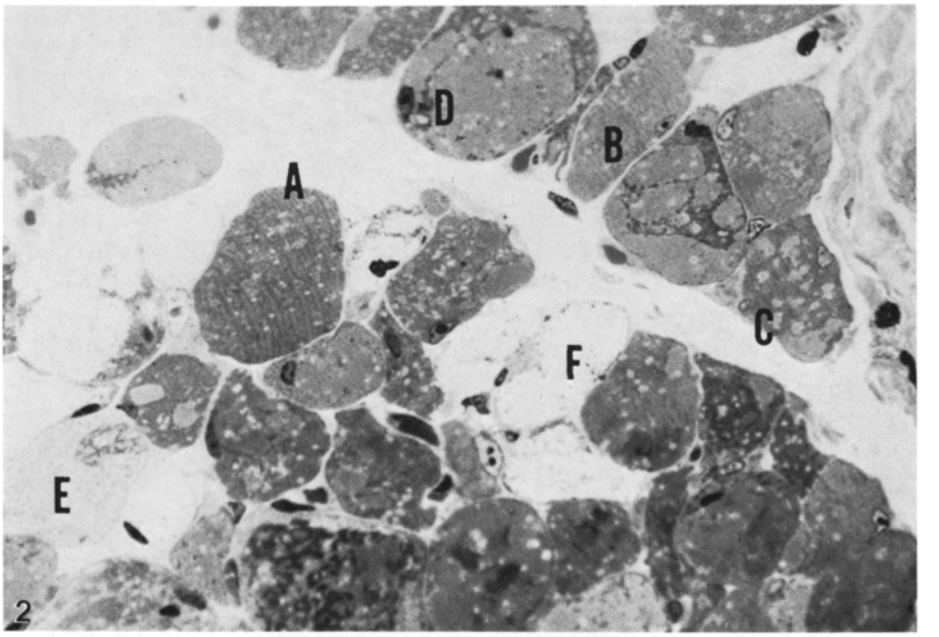
Stage D. Masses of glycogen separate remaining myofibrils or fill fiber cross sections. Metachromatic granules or patches are visible within some glycogen masses.

Stage E. This stage is characterized by dissolution of the glycogen previously accumulated. The glycogen granules may be pale and linked into networks. Broken vacuole membranes may be numerous. Mitochondria often look normal.

Stage F. End-stage fibers look empty, except for sarcoplasmic remnants. Glycogen has been removed except for occasional membrane-enclosed

Fig. 2. Section of muscle from infantile acid maltase deficiency, embedded in epon and stained with toluidine blue, to show the light-microscopic appearance of fibers at different stages during the progressive loss of structure, from stage *A*, almost normal except for some glycogen vacuoles, to stage *F*, empty-looking fibers with remnants of cytoplasm and traces of glycogen. Letters identify some fibers according to the staging list presented in the text. × 575

Fig. 3. This electron micrograph illustrates essentially normal myofiber architecture, except for enclosed glycogen vacuoles scattered between the myofibrils (stage A). There is no excess glycogen free between myofibrils. Shown are two glycogen vacuoles with intact membranes, also mitochondria, lipid, sarcoplasmic membranes, and myofibrils with A, I, and Z bands. Note that the adjacent fiber, bottom, is nearly empty except for sparse glycogen and some debris adhering to the inner surface of the sarcolemma. × 18,000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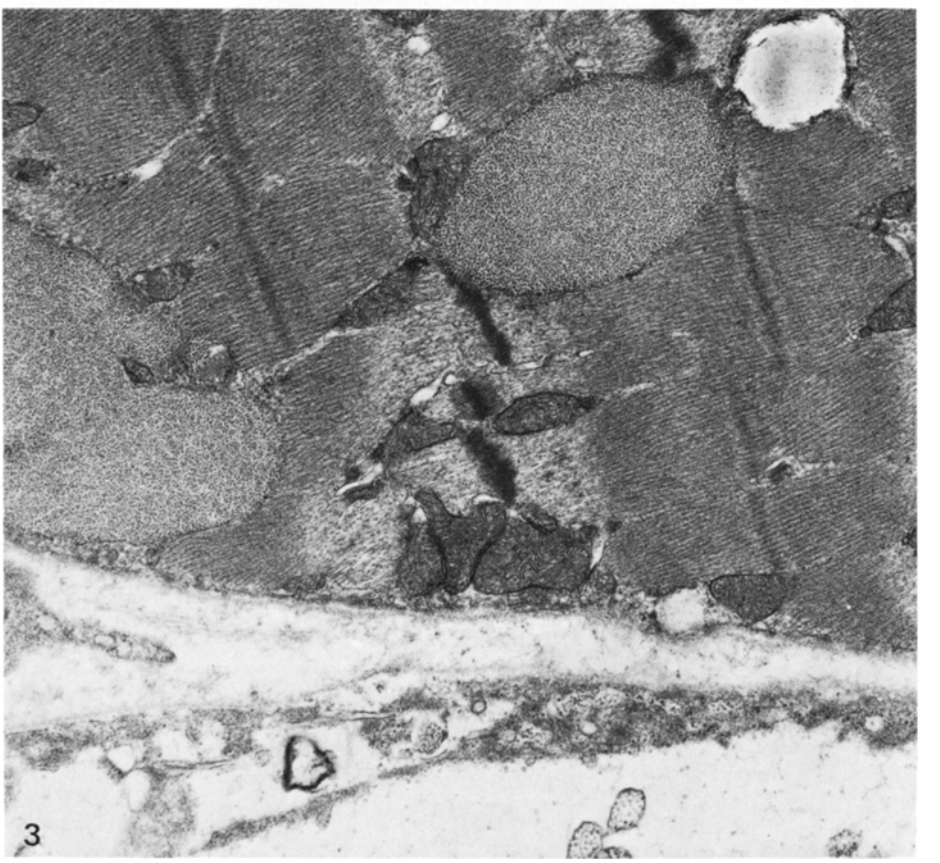
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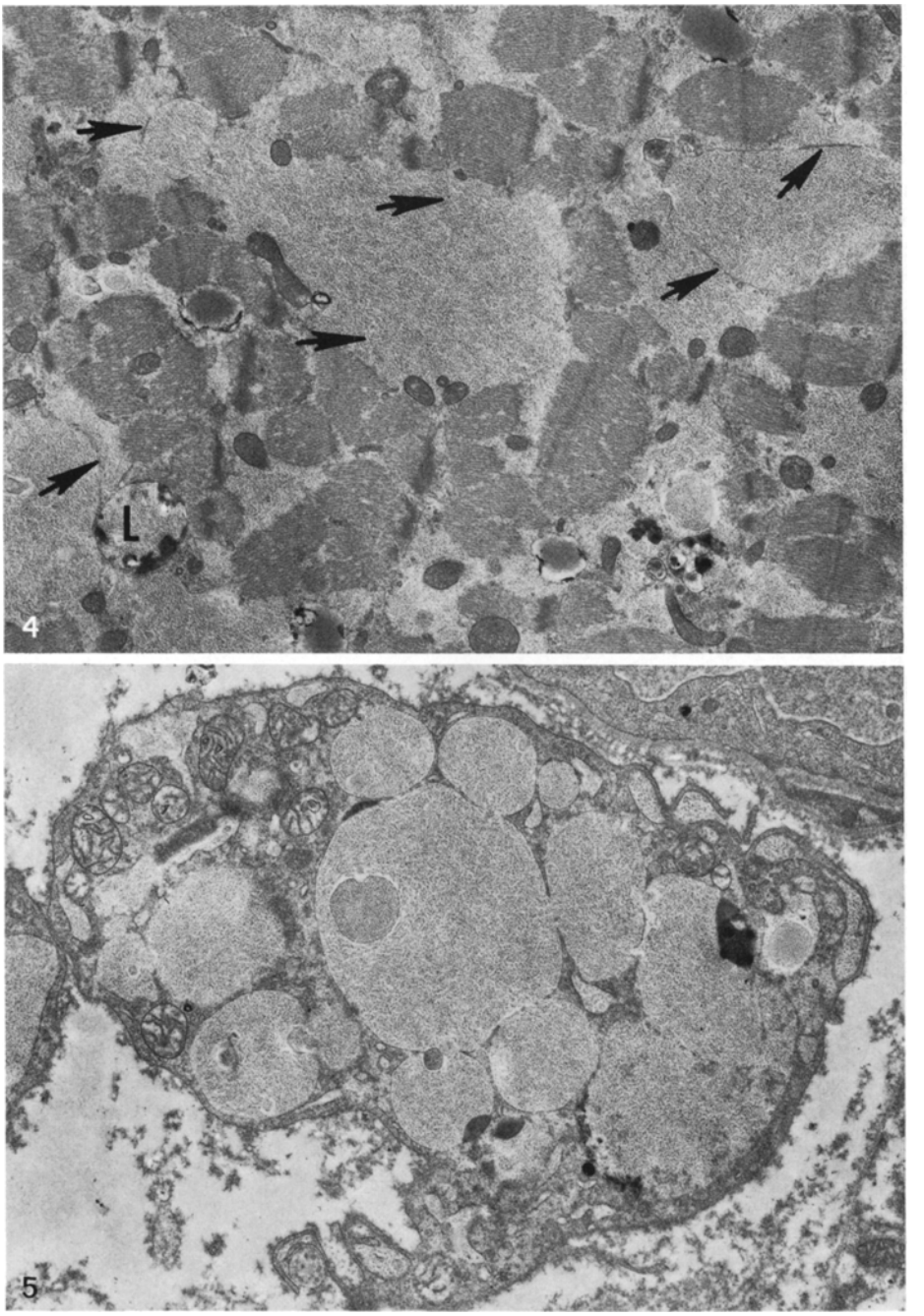


Fig. 4. Glycogen accumulations with associated broken membranes (*arrows*) are seen between myofibrils of a stage C muscle fiber. Broken membranes in the central glycogen lake are thin and have glycogen on both sides, so are not easy to see. A new secondary lysosome (*L*), is recognized as a circular membrane-profile enclosing glycogen and associated dense granules. This muscle fiber can be compared with the macrophage in Fig. 5. × 10,200

Fig. 5. A macrophage, at the same magnification, included for comparison with Fig. 4. Proportionately more of the cross-sectional area is occupied by glycogen in this macrophage, but the membranes remain itact, mostly, in contrast to the muscle fiber in Fig. 4, in which the membranes of larger glycogen masses are broken. × 10,200

spheres or sparse granules adhering to debris under the sarcolemma. Adjacent satellite cells are relatively normal.

In light micrographs of epon-embedded tissue stained with toluidine blue, almost all muscle fibers appear severely altered, many are empty, and some are filled with glycogen rather than with contractile machinery. Stages as in the list above are labeled in Fig. 2, in which a striking diversity can be seen. No fibers without glycogen vacuoles are seen. After osmication, the glycogen accumulated in fibers is metachromatic, with density ranging from a dark bluish red through lighter red, pink, pale pink, and barely detectable pink to apparently empty. Bright red "metachromatic" granules or areas are also metachromatic before osmication (Schnabel 1971; Wolfe and Cohen 1968; Zellweger et al. 1955). For contrast, with similar staining, glycogen accumulations in phosphorylase deficiency stain an orthochromatic blue.

By electron microscopy, no early-stage muscle fibers without glycogen in enclosing membranes are seen. The earliest stage (A), most like normal muscle from infants, is shown in Fig. 3, in which the only abnormality is membrane-enclosed glycogen spheres between the myofibrils.

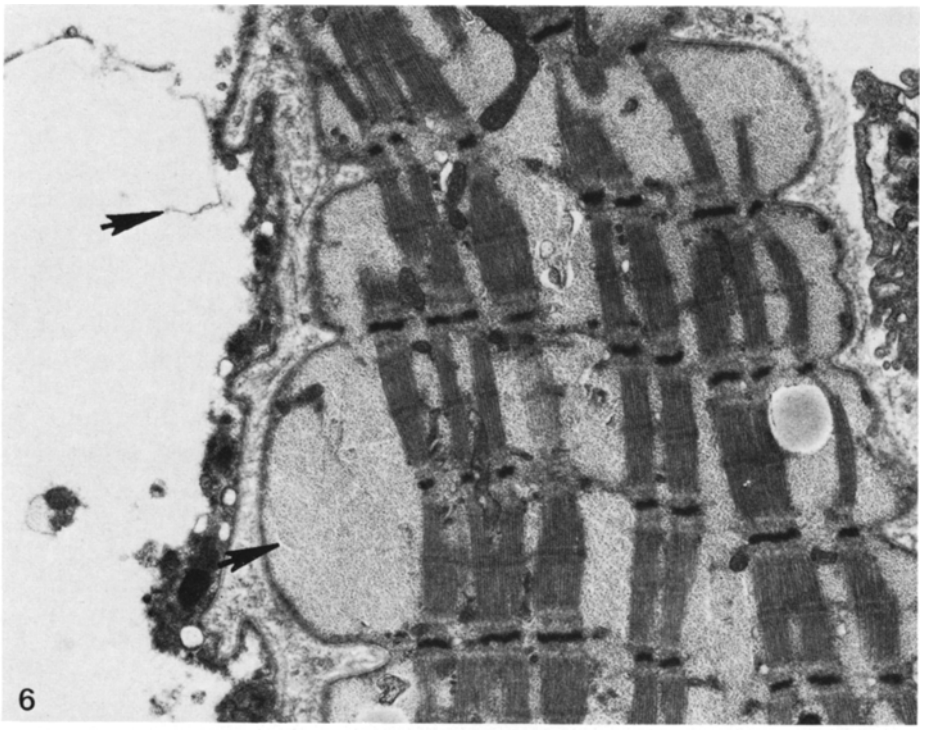
Broken lysosomal membranes are seen at all stages after stage A (Figs. 4, 6, 7, 8). Intact enclosing membranes are seen mostly around smaller glycogen-containing lysosomes or those assumed to be recent because of associated dense particles presumed to have originated with primary lysosomes. Figures 4 and 5 permit a comparison of a muscle fiber and a macrophage, while Figs. 6 and 7 show some of the range of appearance of masses of "free" glycogen and glycogen with enclosing or associated membranes. While the area illustrated in Fig. 6 shows few membranes present, another area of this fiber, in the same section, shows membrane profiles much like those in Fig. 7, a glycogen-filled fiber in which the whole cross section shows a relatively high density of membranes. In several fibers in Fig. 8, characteristic broken membranes are seen.

The proliferation of lysosomes in this disease is indicated by numerous acid phosphatase-positive granules seen by light microscopy. The ultrastructural equivalent to these granules are new secondary lysosomes, circular profiles (Fig. 4) containg glycogen and dense granules presumed to originate from primary lysosomes. In normal muscle, lysosomes are rarely seen either by histochemical methods or electron microscopy.

In stage E fibers, along the edges of myofibril material apparently undergoing dissolution, fine granules are present within the region of microfilament overlap of the A band, as shown in Figs. 9, 10. Since these fine granules are about the same size as glycogen granules, the silver proteinate stains for carbohydrate were applied, as in Fig. 11. Granules in about the same position in similar myofibrillar areas as in Figs. 9, 10 stain with PA-TSC-SP.

Discussion

The lysosomal rupture hypothesis specifies that the membranes of abnormal storage lyosomes in infantile AMD are ruptured by stress related to in-



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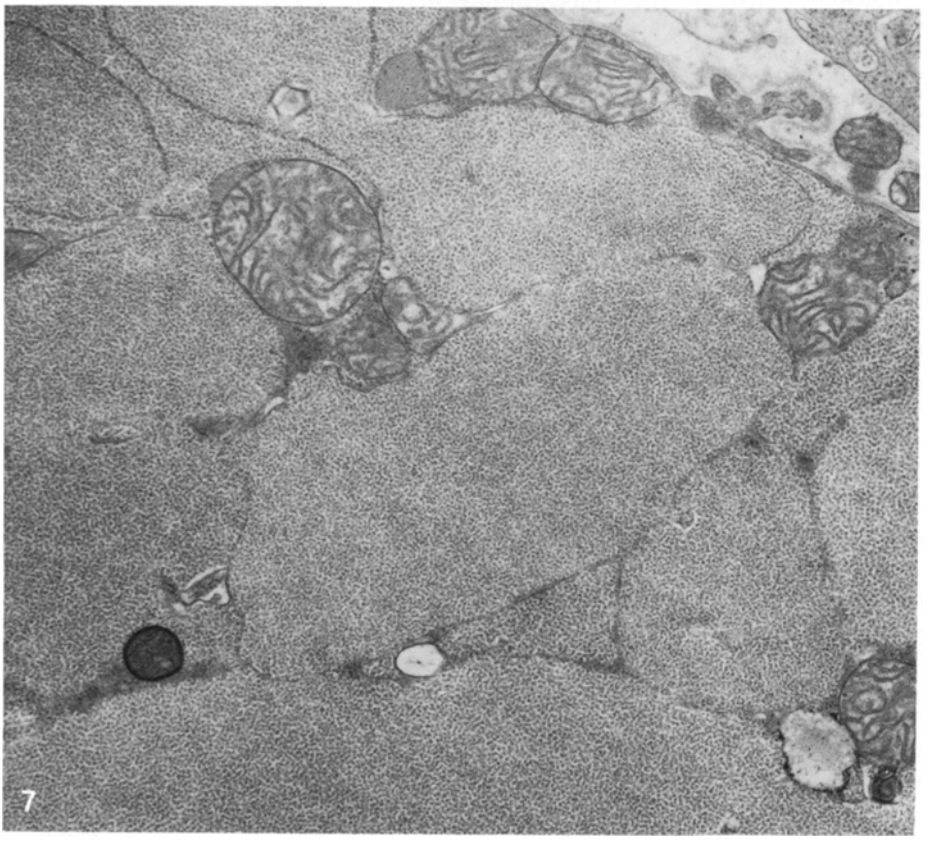

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creased vacuole size and limited space within muscle fibers and that this rupture is related to the muscle cell destruction that characterizes this disease. The results of this study support the hypothesis. Membranes are ruptured in muscle fibers to a much greater extent than in macrophages and other cells. Muscle fibers are also damaged to a much greater extent than other cells.

The membrane barrier, an essential element in concepts of lysosomal disease (Baudhuin et al. 1964; Hers 1963, 1965, 1973; Hers and de Barsy 1973), has been visualized in previous ultrastructural studies of muscle (Cardiff 1966; Engel 1970, 1973; Garancis 1968; Hudgson and Fulthorpe 1975; Hug et al. 1966; Martin et al. 1973; Sarnat et al. 1982; Schotland 1973). The residual broken membranes in muscle fibers visualized in this study and by earlier workers (Cardiff 1966; Garancis 1968; Hudgson and Fulthorpe 1975; Martin et al. 1973) are direct evidence of membrane rupture. Hudgson and Fulthorpe (1975) stated, "Most of the glycogen is localized in the sarcoplasm rather than in vacuoles although some of the former may derive from rupture of over-distended vesicles. Certainly large amounts of redundant membrane can be seen within the sarcoplasmic glycogen deposits suggesting that this might have occurred."

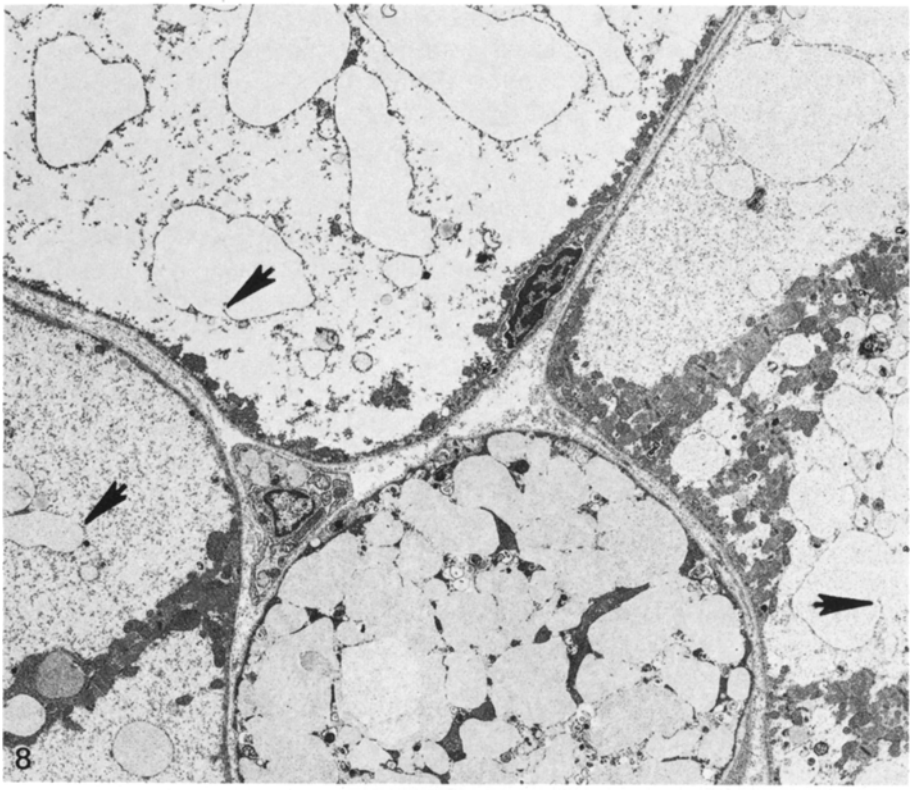
Cardiac muscle (Cardiff 1966; Hug 1972) also shows large amounts of free glycogen and broken membranes in infantile AMD and lacks space for accumulation of enlarged lysosomes.

If we compare cells that exhibit ruptured lysosomal membranes with cells in which lyososomes are mostly intact, the damage to muscle is striking and much more dramatic than changes in liver (Baudhuin et al. 1964; Cardiff 1966; Garancis 1968; Hers 1965; Hug 1972; Hug and Schubert 1967). Cells without the structural specializations of muscle can expand as storage material is accumulated. Non-muscle cells also lack the physical movements and changes in rigor that accompany contraction of muscle. In liver, macrophages, epithelium, central nervous system, stallite cells, and cells of other organs, glycogen of normal density is enclosed within intact membrane barriers (Cardiff 1966; Garancis 1968; Hug 1972; Martin et al. 1973). An unusual naked aggregate of glycogen in a liver cell was shown by Hug et al. (1973), possibly produced by a rare breakage of the membrane barrier.

A sequence of fiber degeneration with empty or nearly empty fibers as the end point has not previously been proposed. In previous ultrastructur-

Fig. 6. The field shown (stage C) was selected to represent an appearance as close as possible to that seen in other glycogen storage diseases. Much glycogen, not enclosed by membranes, separates myofibrils and is beneath the surface. In this section, in another area of this same fiber, an accumulation of glycogen was almost fully enclosed by membranes, as in Fig. 7. To the left is an empty stage F fiber. Both the fiber with glycogen and the empty fiber exhibit a few broken membranes (*arrows*). × 10,200

Fig. 7. In this fiber (stage D, glycogen-rich) the glycogen fills the fiber except for a few mitochondria. Note enclosing membrane profiles which suggest that glycogen shown accumulated within vacuoles. This section was selected as the best example seen of a filled fiber in which almost all accumulated glycogen was within membrane profiles. To upper right, a few organelles are free between fibers. × 18,000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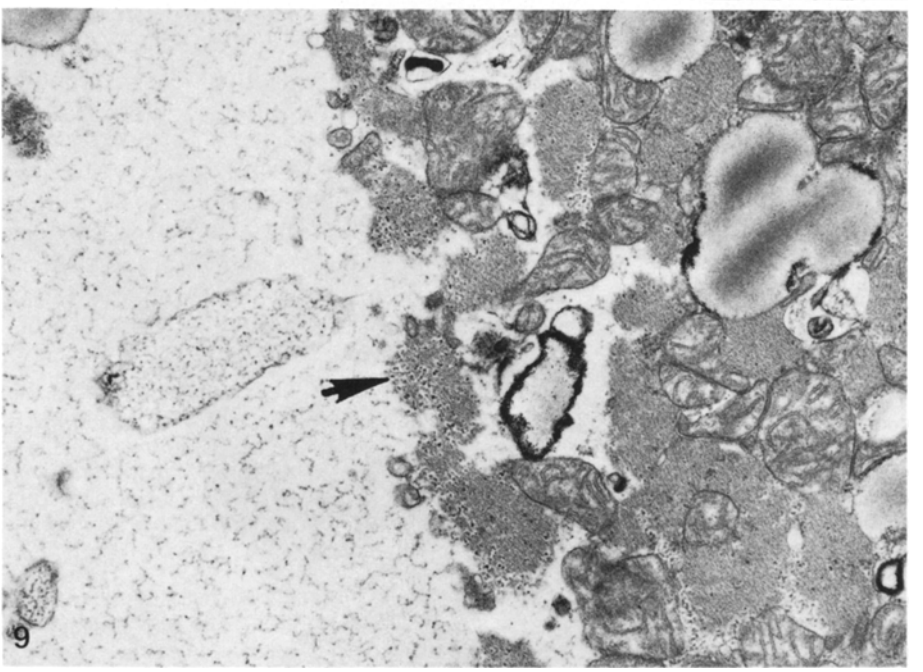
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al studies of muscle in infantile AMD (Cardiff 1966; Garancis 1968; Hug et al. 1966; Hudgson and Fulthorpe 1975; Martin et al. 1973), the glycogen-filled fibers have been regarded as end stage. For example, Cardiff (1966) reported that all skeletal muscle cells contained massive glycogen deposits.

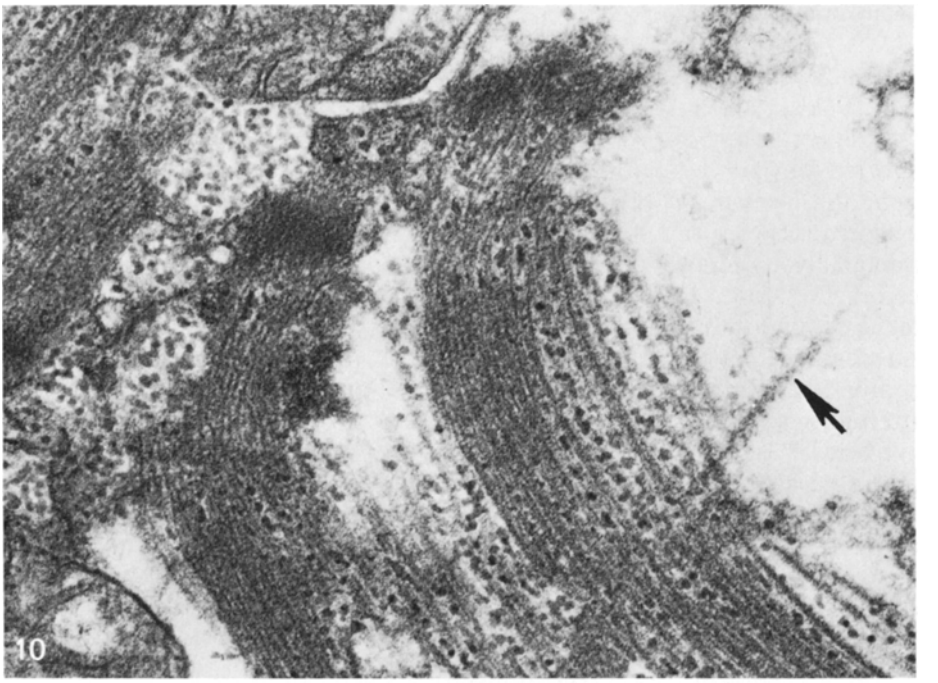
From the evidence presented herein, based on material fixed in glutaraldehyde and processed for electron microscopy, there is no indication that empty fibers contained glycogen that was extracted during fixation or processing. All stages in sequential digestion of glycogen were visualized with toluidine blue staining of light microscopic epon sections in different fibers and correlated with electron microscopy to confirm that the material in the fibers is fixed and visualized.

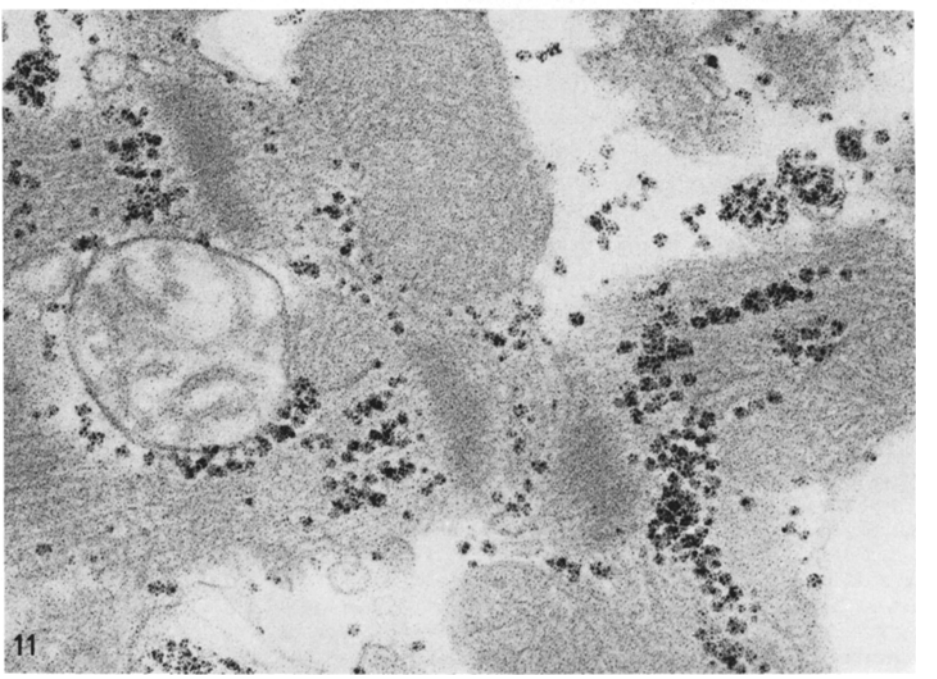
In muscle fiber destruction, mitochondria are relatively late to change. In infantile AMD, no decrease in fiber volume is noted after the hypertrophy associated with glycogen accumulation and a few remaining reticulately connected glycogen granules can be extensively separated and distributed evenly throughout relatively large spaces. There is no indication of attempted regeneration. Satellite cells are unchanged except for glycogen vacuoles smaller than those of macrophages (Engel 1973; Hudgson and Fulthorpe 1975). No phagocytosis or inflammation is seen.

Why are the myofibrils lost or decreased in size and of abnormal structure? At least two mechanisms of myofibril loss may apply. First, the rupture of the lysosome releases not only glycogen but also the contained enzymes. These enzymes could act to digest the myofibrillar material. There is stimulation of lysosome production, as staining of frozen sections reveals numerous acid phosphatase granules (lysosomes) in each fiber, compared to normal muscle in which none are seen, a result also confirmed by electron microscopy. Second, the muscle may well lose the ability to contract. If denervated muscle or muscle in type II atrophy does not contract, the muscle atrophies and the fibers also undergo atrophy and loss of myofibrillar material. There is differential centrifugal evidence (Canonico and Bird 1970) and morphological evidence (Engel 1970, 1973) that the membranes with lyosomal activity are part of the T-tubule and sarcoplasmic reticular system, not normally visible as lysosomes with cytochemistry or electron microscopy. If T-system membranes are used to segregate accumulating glycogen, signals for contraction may not reach the myofibrils and regular contraction may not occur.

Fig. 8. Electron micrograph of portions of one end-stage (F) fiber, two fibers of late stage E, and one fiber (early stage E) containing much glycogen with associated membranes. Remaining sarcoplasmic material crosses the spaces of the fibers to left and right. Each fiber contains broken membranes and some breaks are indicated by *arrows*. With toluidine blue, the upper fiber was colorless, those to left and right were barely detectable pink, and the bottom fiber was light pink. ×3,795

Fig. 9. To the left, in a stage E fiber, is space occupied by remaining scattered glycogen granules. Myofibrils with mitochondria and lipid are visible to the right. In the myofibrils bordering the space are dense granules, of about the size of glycogen granules, embedded within the myofibrils (*arrow*). ×18,000





Figs. 10, 11. Comparable views of myofibrils undergoing dissolution, stained with uranyl-lead and with Periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP). **Fig. 10.** With uranyl-lead, there are clearly glycogen granules with the mass of myofibrillar material, but there also darker granules of about the same size that were thought to be possible indicators of digestive change in the myofibrils. At the *arrow*, a remnant of the M band extends. This material, which connects the thick filaments in the middle of the A band, was apparently left after filaments were lost. **Fig. 11.** the PA-TSC-SP stain reveals that granules staining as glycogen does are in much the same spaces as those granules presumed to be possible products of digestion. × 57,000

This report does not deal in depth with two specific features of muscle in infantile AMD that lead some investigators to postulate additional enzyme defects or extralysosomal factors to account for the changes. These are, first, the massive accumulation of nonenclosed glycogen in the sarcoplasm and, second, metachromatic material, sometimes interpreted as acid mucopolysaccharide, in glycogen filled fibers. These topics are considered in detail by Griffin (1983a, 1983b). Hers and de Barsy (1973) concluded that, even without specifically accounting for these two features, the coexistence of two inborn enzyme deficiencies was extremely unlikely.

In conclusion, the role of lysosomal rupture in muscle destruction can be inferred by comparing muscle fibers containg intact abnormal lysosomes with fibers containing ruptured membranes in which the lysosomal contents have been dumped into the cytoplasm. Muscle fibers with intact lysosomes exhibit otherwise normal ultrastructure. Muscle fibers with ruptured membranes and glycogen in the cytoplasm exhibit various stages of fiber destruction.

From evidence now available, the simplest proposal to account for the relentless deterioration of muscle in infantile acid maltase deficiency is the enzyme deficiency identified by Hers (1963, 1965), with one addition. As specified by the lysosomal rupture hypothesis, abnormal storage lysosomes do rupture in muscle and this seems highly likely to contribute to muscle fiber destruction. The profound weakness that characterizes infantile acid maltase deficiency seems related to the loss of contractile machinery.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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