Intralysosomal cystine crystals in cystinosis

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Electron microscopic study of conjunctival biopsies from members of one sibship each of nephropathic and benign cystinosis showed polymorphic cystine profiles abundantly distributed in fibroblasts and histiocytes. These crystalline spaces measured from 0.2 to 2μ and were delineated marginally by intact cellular membranes. Small crystalline aggregates were found in acid-phosphatase-positive osmophilic dense bodies and they appear to be the earliest manifestation of intracellular deposition of cystine. This study suggests that there are striking morphologic similarities in the two forms of cystinosis at the cellular level and supports the suggestion that both may represent disorders in cystine metabolism by a subcellular organelle of lysosomal type.

Key words: cystinosis, nephropathic cystinosis, benign cystinosis, conjunctiva, crystals, lysosome, cystine.

Cystinosis is a familial disorder characterized by an accumulation of cystine crystals in various organs. Two clinical forms are recognized; nephropathic cystinosis and benign cystinosis. Nephropathic cystinosis has its onset in infancy and is accompanied by fever, dehydration, polyuria, polydipsia, growth retardation, and rickets. Death from uremia generally occurs within the first decade. The presence of crystals in pathologic specimens of renal tissues suggests that the deposits themselves are damaging to the kidney parenchyma. Benign cystinosis is distinguished by the absence of any detectable glomerular or tubular dysfunction and renal cystine crystals are not present.

The primary defect in this disorder is unknown. The accumulation of the crystals is related to a high intracellular concentration of cystine. The free cystine content of leukocytes and cultured skin fibroblasts from patients with nephropathic cystinosis is approximately 100 times that of normals. The intracellular cystine values obtained from cells of patients with the benign form of the disease are also elevated, but they are only 40 to 50 times higher than in normals. Furthermore, leukocytes and fibroblasts from parents (heterozygotes) of children affected with the nephropathic type usually contained 5 to 6 times the normal content of free cystine. This finding has made possible the biochemical identification of the heterozygote in this autosomal recessive disease.

In both forms of cystinosis, the homo-


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Fig. 1. Abundant crystalline profiles are seen in the cytoplasm of the histiocyte. Small crystals are found in lysosomes. (x25,000.)

Fig. 2. Higher magnification of a portion of Fig. 1. Crystals are delineated by membrane which is identical to that of lysosome. Small crystals are seen in the lysosome. (x60,000.)

geneous, fine tinsel-like deposits of the cornea and the conjunctiva are sufficiently suggestive to enable the diagnosis to be made by slit lamp examination. Conjunctival crystals from affected patients have been identified as L-cystine and are identical to those crystals found in other tissues.

Biochemical evidence suggests that the increased intracellular cystine may be compartmentalized in a subcellular organelle. For this reason, bulbar conjunctival biopsies, which are easily obtained, were taken for electron microscopic study in the hope of visualizing cystine crystals within a subcellular component.

Material and methods

Conjunctival biopsies were performed on twelve members representing two families of
which three patients had nephropathic cystinosis and two had benign cystinosis.

The biopsy specimen was fixed in a 4 per cent glutaraldehyde solution for 24 to 36 hours, pH 7.2, a 0.15M phosphate buffer, and in a 1 per cent osmium tetroxide solution in the same buffer for 1½ hours. After dehydration in graded ethanols, the tissue was embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate.

For acid phosphatase studies, the biopsy specimen was trimmed to less than 1 mm. square and fixed for one hour in a 2 per cent glutaraldehyde solution (4° C.) containing 0.1M phosphate buffer at pH 7.2. After a 24 hour wash with the above buffer (4° C.), the tissue bits were divided and transferred into corresponding media for the Gomori test and control. The medium contained 0.05M acetate buffer at pH 5.6, 0.004M lead nitrate, and 0.01M β-glycerophosphate. The latter was omitted in the control medium. All tissues were incubated for 30 minutes at 37° C., and transferred to 1 per cent osmium tetroxide in 0.1M phosphate buffer at 4° C. for one hour. They were dehydrated in graded ethanols and propylene oxide and embedded in Araldite. Thin sections were examined in an electron microscope.

Results

The subepithelial connective tissue appeared thickened and the number of fibroblasts and histiocytes was increased. Poly-
Fig. 4. Acid phosphatase reaction. Crystal profile is seen within a membrane-bounded dense body. Dark speckling is site of acid phosphatase activity (arrow); note that activity is not homogenous throughout the body. Bar represents 1 µ.

Morphologic crystalline profiles of squares, rhomboids, oblongs, and hexagons were found abundantly distributed in these cells (Fig. 1). Most of these crystalline spaces measured from 0.2 µ to 2 µ and were delineated marginally by intact intracellular membranes. Small crystalline aggregates were confined to osmophilic dense bodies which had the characteristic appearance of lysosomes (Fig. 2). This impression was confirmed by the presence of acid phosphatase activity, manifested by electron-dense precipitates, in these crystalline-containing round cytoplasmic bodies (Figs. 3 to 5). The evidence of enzyme activity was also seen surrounding many of the membrane-limited large crystals.

The intralysosomal crystals appear to be the earliest manifestation of intracellular deposition of cystine. It seems possible that the observed large crystals represent growth products initiated from the membrane-limited small crystals. Grossly abnormal and degenerated cells contained an increased number of large crystals.

Some empty crystalline profiles seemed
to contain a granular proteinaceous substance, apparently left behind after the cystine has been dissolved during tissue processing. Crystalline forms were not found in the extracellular spaces. The blood vessels were normal and free of crystals. The pathologic changes of the conjunctival tissue were similar in the two forms of cystinosis.

No crystalline material was observed in any subcellular organelle in the conjunctiva of heterozygous carriers. The only unusual finding in them was a central lucency in many of the normally electron-dense lysosomes of histiocytes. Whether this lucency represents the site of cystine accumulation or whether it represents a normal variation in the density of lysosomes is unknown.

Discussion

Although the primary defect in cystinosis has not yet been identified, the possibility exists that this disorder may arise from a defect of cystine metabolism in the organelles in which the cystine crystals are stored. The demonstration of acid phosphatase activity around the membrane-limited crystalline profiles supports the morpho-
logic impression that these subcellular structures are of lysosomal origin. Further evidence will be required, however, before differentiation of these lysosome types to phagosomes or autophagosomes could be made. The presence of crystalline intralysosomal inclusions in conjunctival tissue appears to represent a more serious generalized pathologic process in that similar findings have recently been reported in a lymph node biopsy from a case of nephropathic cystinosis. Our observations are at variance with those of Morecki and co-workers, who suggested that the defect may reside in the mitochondria. The morphologic evidence of intralysosomal crystals suggests but does not necessarily preclude derangement of other metabolically related organelles such as the endoplasmic reticulum.

The increased intracellular cystine observed in both forms of cystinosis has been shown by Schneider and co-workers to localize in the granular fraction of blood leukocytes and skin fibroblasts by subcellular fractionation. The present study does not confirm but strongly implies that this cystine-rich fraction might be lysosome-related.

The major difference between the nephropathic and benign forms of cystinosis is the absence of renal and retinal defects in the latter type. The present study suggests that there are striking morphologic similarities evident in the two disorders at the cellular level. Whether or not the two forms represent variations in the severity of expression of the same underlying primary metabolic defects remains to be demonstrated.

REFERENCES


