Reovirus 1–induced hydrocephalus of mice has been noted by Kilham and Margolis (*12*) in a study carried out principally in hamsters. Details of the pathology, virology, morbidity rate, and time taken to clinical onset of the disease in mice were not specifically mentioned. Hamsters inoculated neonatally with reovirus 1 have been found to develop noncommunicating hydrocephalus (*12, 19*) beginning 8 to 10 days after inoculation. At this time infectious virus was still persistent in brain and liver. The pathogenesis of the disease involved the ependymal cells lining the aqueduct of Sylvius (*20*).

The mean time span of 109 days between inoculation of the virus and manifestation of hydrocephalus is a noteworthy feature of the present series of experiments. It is comparable with that observed for LGV-infected mice (*6*) and greater than the maximum time recorded for myxovirus- (*4*) and polyoma virus–induced hydrocephalus (*5, 10*).

Induction of hydrocephalus in animals inoculated by routes other than the intracerebral one is known only for polyoma virus (*5*) and reovirus (*12*). To our knowledge, the disease has not been reported in any experimental animal after oronasal infection with virus.

The induction, via the oronasal route, of hydrocephalus in mice by such a ubiquitous virus as the mammalian reovirus means that similar induction could easily occur under natural conditions in many species, including man. The search for such a cause is complicated by the long latent period before clinical disease and the associated disappearance of infectious virus (phenomena now increasingly recognized as the outcome of virus-host interaction). It may be an important clue that this capacity for inducing hydrocephalus is restricted to only one of the three reovirus types.

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20. Pathological studies being carried out in our own series by Dr. Byron Kakulas will be reported in a separate communication.
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## Human Cystinosis: Intracellular Deposition of Cystine

Abstract. *Membrane-limited inclusions were found in the cytoplasm of cells of the rectal mucosa, leukocytes, and cultured fibroblasts from two humans with cystinosis. Most of the inclusions contained amorphous material, presumably cystine. In cells of the rectal mucosa the material appeared frequently crystallized. This was rarely seen in leukocytes, and never in cultured fibroblasts. The fact that acid phosphatase could be demonstrated consistently in the organelles responsible for sequestration of cystine indicates that they are lysosomes.*

Cystinosis, a heritable disease of childhood, is characterized by cellular accumulation of cystine (*1*). In some cells, primarily those of the reticuloendothelial system, this accumulation leads to crystal formation which is readily visible with either the light or electron microscope. In other cells, such as leukocytes or fibroblastic cell lines derived from cystinotic children, no crystal formation has been observed with light microscopy although biochemical analysis shows that both types of cells have a high amount of cystine (*2, 3*). Indirect evidence has suggested that the lysosome is the organelle of storage in cystinotic fibroblasts (*3*). Direct evidence of this has been presented for crystals in reticuloendothelial cells where the limiting membranes showed acid phosphatase activity (*4*).

Recently, differential centrifugation of homogenates of leukocytes from cystinotic patients has revealed the cystine to be associated with the cell fractions high in lysosomal enzyme activity (*5*). Calculations of specific gravity from the density gradients used suggested that the cystine was very likely not crystalline but in an amorphous form. We now present electromicroscopic evidence that in leukocytes, fibroblasts, and other cells of cystinotic subjects that do not reveal crystal formation, cystine is stored in amorphous form compartmentalized in cell organelles consistent with lysosomes.

Cells of the lamina propria of the rectum (Fig. 1) in cystinotic children are particularly rich in stored cystine

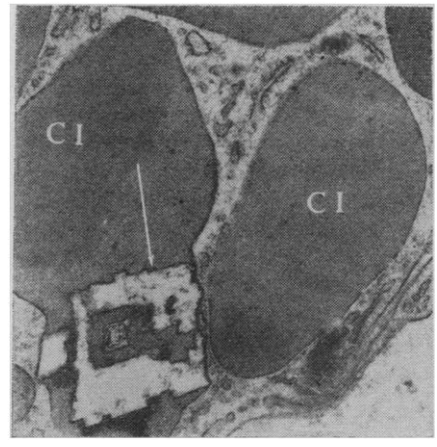


Fig. 1. Rectal biopsy from patient J.E. The tissue was fixed in 1 percent buffered osmic acid, dehydrated in ethanol, and embedded in epoxy resin. Sections were doubly stained with uranyl acetate and lead citrate. The cytoplasm of the cell of the lamina propria contains large inclusions (*CI*). Some of the material has crystallized (arrow), whereas in other areas it appears amorphous (× 36,750).

(*6*). Although crystals are readily evident most of the cystine seems to exist in amorphous form. In some of these large membrane-limited inclusions the material seems to have crystallized, whereas in others this is not evident. Since the crystal formation is considered pathognomonic for the disease, it is reasonable to suggest that all material in these inclusions is L-cystine and that crystallization is not inevitably a condition for its storage. Amorphous accumulation has been suggested before (*7*). In leukocytes obtained from another child, crystals were evident only occasionally (Fig. 2A). Practically all white cells had inclusions of various sizes, which appeared as amorphous grayish material. After the cells were stained for acid phosphatase by the method of Gomori practically all these inclusions showed enzyme activity (Fig. 2B). The association of acid phosphatase activity with the inclusions would indicate that they are lysosomal in nature (*8*).

Fibroblast cell lines derived from the skin of cystinotic children frequently contained inclusions surrounded by a double limiting membrane. Amorphous material was evident in these inclusions, although crystallization was never observed (Fig. 2C). The cytoplasmic inclusions were never seen in fibroblast cell lines derived from normal people. After staining for the presence of acid phosphatase all these inclusions showed enzyme activity (Fig. 2D). Where contents of the inclusions were absent the membranes were stained intensely.

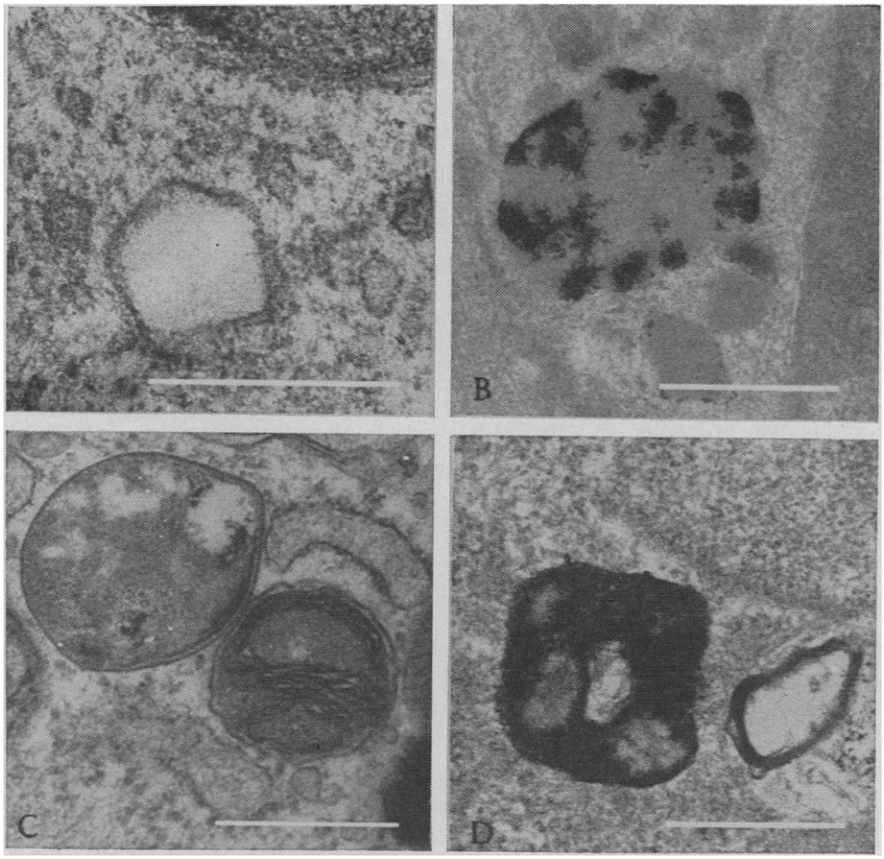


Fig. 2. (A and B) Leukocytes from patient B.K. Blood was collected in a heparinized syringe. Cells of the buffy coat were fixed in 3 percent glutaraldehyde buffered with cacodylate. One half of the cells were stained for acid phosphatase by the method of Gomori (*8*). The other half served as control. Stained and control cells were fixed in 1 percent osmic acid, buffered with barbital, dehydrated in ethanol, and embedded in epoxy resin. Sections of control cells were stained as above. Cells stained by the Gomori method were observed without further increase in contrast. (A) A crystal in the cytoplasm of a small lymphocyte (× 73,500). (B) Cytoplasmic inclusion in a leukocyte containing amorphous material and showing acid phosphatase activity (× 52,500). (C and D) Cultured fibroblasts from skin biopsy from patient J.E. The fibroblast cells were cultured in McCoy's medium (5a modified) supplemented with 15 percent fetal calf serum. Cells from tissue culture passage number 4 were embedded by standard methods; cells from passage number 7 were stained for acid phosphatase as described above. (C) Cytoplasmic membrane-bound inclusions containing amorphous material. No crystals are evident (× 52,500). (D) Cytoplasmic inclusion showing acid phosphatase activity and containing amorphous material. Membrane of an empty inclusion stained for acid phosphatases (× 52,500). The bar represents 0.5 μm.

At present chemical data are not sufficient for us to determine whether crystal formation is directly dependent on the quantity of cystine in the various tissues. Assay for cystine levels with an automatic amino acid analyzer in rectal mucosa biopsy where crystals are abundant revealed 113.3 μmole of half cystine per gram of protein; in cultured fibroblasts from patient J.E. the value was 18.7 μmole per gram of protein. Normally tissue cystine is below the limits of accurate detection by this method (*3*).

Our results present evidence that cystine does not have to be present in cells in crystalline arrays and furthermore that in all cells investigated it is compartmentalized. The regularity with which acid phosphatase activity could be demonstrated in these compartments in leukocytes as well as fibroblastic cell lines from cystinotic patients suggests that, as indicated previously, the lysosome is the organelle responsible for storage (*5*). It seems reasonable to suggest that crystal formation of cystine is an end product of its accumulation and that cells that exhibit numerous and large crystals do so either because they represent a preferential environment for storage or because they replicate slowly, thus allowing for excessive accumulation.

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