differences in the morphology of the system of trabeculae, as visible in the SEM. This assumption is strengthened by comparing the corresponding structures of holothuroids, crinoids, asteroids, and ophiuroids, examples of which were also photographed in the SEM.

While the outer shapes of the trabeculae are generally rounded, the fracture surfaces of single trabeculae are in part strictly planar, and their position with regard to the plate coordinates and the crystal axes known from the x-ray studies indicates that they are rhombohedral planes $\{10\bar{1}1\}$ (Fig. 4). The areas showing these cleavages are up to 0.1 mm in diameter and thus within the range of optical microscopy, especially in the specimens with relatively little "pore volume." However, the fracture surfaces show mainly what is usually called "conchoidal fracture" (Fig. 2c). This is very unusual for calcite, fragments of which are normally completely bounded by cleavage faces, and it may explain why echinoid spines and plates show no preferred macroscopic fracture surfaces. A possible explanation is seen in the area marked by an arrow in Fig. 1b as well as in Fig. 4—on this cross section a zonar structure is visible. Nothing is known as yet about the chemical or crystallographic differences (or both) of these zones, which may be growth structures. It is likely that this layering gives rise to the unusual morphology of fracture surfaces of the trabeculae.

For evaluation of relations between the crystallographic symmetry and the shape symmetry of the biocrystal the lattice position must be fixed in space relative to the organism's morphology. Echinoid plates usually have their *c*-axes either parallel or perpendicular to the plate surface (*10*). In *D. excentricus*, for example, *c* is normal to the plate surface within 1° of approximation. Nine aboral plates were investigated in a juvenile specimen of this species. The plates were left in their mutual orientation relation, that is, the entire bottom of the skeleton was mounted on a Laue camera. Two photographs were taken in such a way that the x-ray beam could pass through parts of two adjacent plates. In spite of an astonishing parallelism of *c*-axes (the bottom in this species is rather flat), the *a*-axes vary considerably in their position, though generally one of them points approximately toward the anus. In one specimen a pair of adjacent plates, lying on the morphological symmetry plane, show the two lattices rotated with respect to each other 60° around the *c*-axes, which approaches a twin relation to a high degree of approximation. This orientation relation is unusual because in some hundred echinoderm skeletal units investigated the crystals were always found to be single crystals without any twinning.

Measurements of lattice orientation were made by using the texture x-ray camera (*6*) because, besides the common cases where *c*-axes are parallel or perpendicular to the plate surface, certain species exist (*4*) in which the *c*-axis is inclined to the plate surface and the angle of inclination varies from plate to plate (*2*). In two plates selected respectively from *Sphaerechinus granularis* and *Strongylocentrotus franciscanus*, having nearly identical *c*-axis orientations with respect to morphology, the *a*-axes differ by a rotation around the *c*-axes of approximately 60°. These examples show the great variety of lattice orientations with regard to shape and symmetry elements of the skeletal units which must be expected in the echinoids. Many more species must be investigated before these measurements can be used successfully for the evaluation of phylogenetic relations.

Recent physiological findings (*10*) are compatible with the assumption, which is offered as a working hypothesis, that the collagen fibers found in the hollow spaces between the trabeculae act chemically as a substance that inhibits further secretion of calcite around them. These fibers, therefore, are assumed to act in an opposite way to the organic envelope, secreted by the cells at an earlier stage, that promotes calcite growth. The layering shown in Fig. 4 seems to indicate changes in the secretion conditions during growth of a plate.

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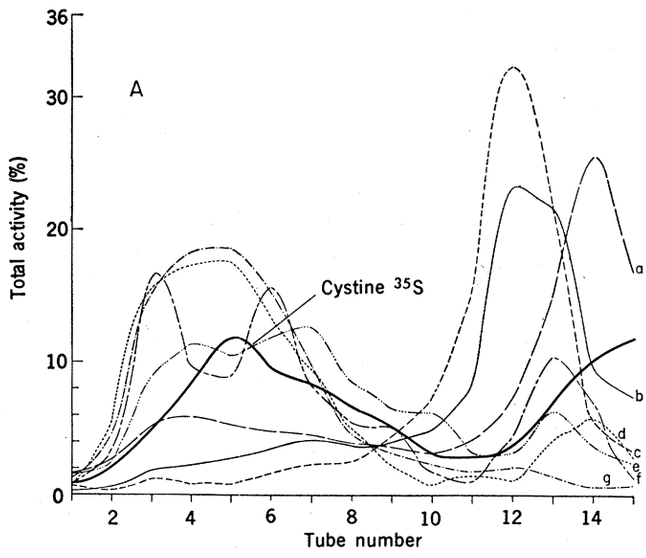
Cystine: Compartmentalization within Lysosomes in Cystinotic Leukocytes

Abstract. *The large amount of cystine compartmentalized in cystinotic leukocytes cosediments in isopycnic sucrose density gradients with dense lysosomal particles, within which it is presumably contained. Such cystine appears to be primarily noncrystalline in these organelles.*

Nephropathic cystinosis, cystine-storage disease of childhood, is a recessive heritable biochemical disorder characterized by a high intracellular concentration of cystine and accumulation of cystine crystals in many organs of the body (*1*). Such accretion within the kidney is associated with renal tubular dysfunction and glomerular damage leading to death in uremia usually before puberty.

The precise subcellular location of the cystine has not been determined. In cystinotic leukocytes and fibroblasts, the



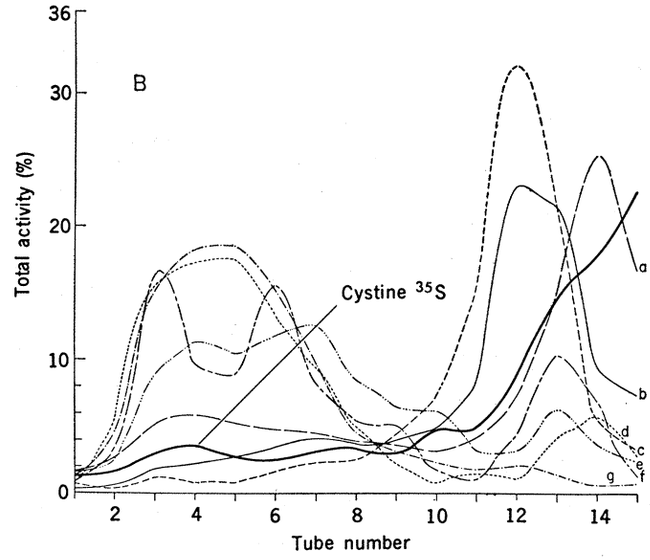
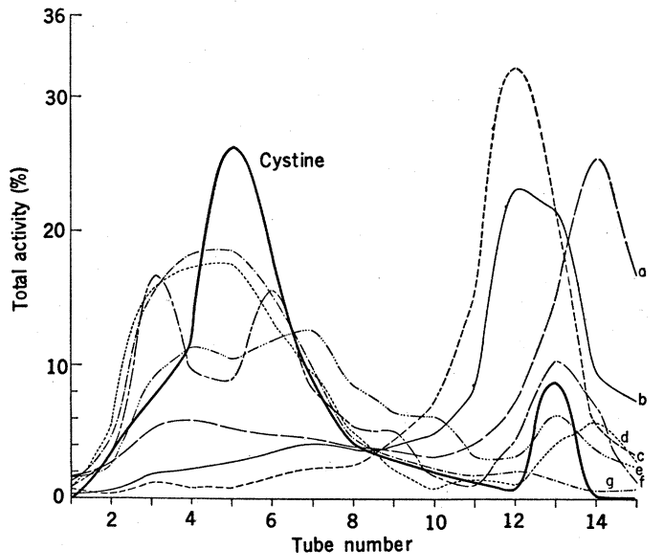


Fig. 1 (above). Distribution of intracellular cystine-^{35}S and lysosomal enzymes in sucrose density gradients after brief exposure to cysteine-^{35}S. (A) Cystinotic and (B) normal leukocytes with cysteine-^{35}S; *a*, protein; *b*, acid phosphatase; *c*, alkaline phosphatase; *d*, α-mannosidase; *e*, *N*-acetyl-β-glucosaminidase; *f*, β-galactosidase; *g*, peroxidase. The curves are averages of several determinations. Fig. 2 (right). Distribution of intracellular cystine and lysosomal enzymes in sucrose density gradient of cystinotic leukocytes. Cystinotic leukocytes were prepared and processed as in Fig. 1, but no incubation with cysteine-^{35}S or neutral red was performed; cystine in each fraction was determined colorimetrically by column chromatography (*2*).



cystine is compartmentalized intracellularly (*2*), and it has been suggested that cystine accumulates in some as yet unidentified organelle that is more susceptible to hypotonic lysis than are lysosomes (*1*). Electron micrographs have demonstrated crystalline images, presumably of L-cystine, within lysosome-like organelles in lymph node and conjunctiva of affected children (*3*), whereas similar photomicrographs of a single intestinal biopsy suggested mitochondria as the site of crystalline cystine storage (*4*).

We now describe distribution of cystine after isopycnic sucrose density gradient centrifugation of the large intracellular cystine pool of cystinotic leukocytes (Figs. 1 and 2). These cells were chosen for analysis as they were conveniently available, and because light and electron microscopic observations (*5*) suggested that most of the cystine compartmentalized within them might be in noncrystalline form.

White cells from cystinotic or normal individuals were prepared by sedimentation from fresh whole blood with 3 percent dextran at 4°C, followed by hypotonic lysis of erythrocytes (*6*). The leukocytes obtained consisted mostly of the mature polymorphonuclear cells that contain most of the cystine in preparations of mixed leukocytes from cystinotic blood (*5*). The cells were first incubated for 45 minutes at 37°C, aerobically, in Krebs-Ringer solution containing 0.054 m*M* cystine-^{35}S (67 mc/mmole, Amersham) maintained in reduced form as cysteine by 0.20 m*M* dithiothreitol; the medium also contained neutral red (5 μg/ml) as a lysosomal marker (*7*). The cells were then washed and disrupted by high-frequency sound (*2*).

After the nuclei were removed by centrifugation at 600*g*, 0.75 ml of the supernatant in 0.25*M* sucrose was layered on top of a 4 ml linear sucrose gradient, density 1.32 to 1.10; the gradients were centrifuged (Spinco SW 39L) at 115,000*g* for 2 hours. Fifteen equal fractions were collected with a needle inserted through the bottom of the tube, and protein and enzyme assays were performed on each fraction (*8*, *9*). Radioactive cystine was separated from 20 μl of each fraction by electrophoresis in 6.8 percent formic acid at 4000 volts for 2 hours; then the cystine areas were cut out, and radioactivity was counted in a scintillation counter.

Gradients of subcellular components of cystinotic and normal cells did not differ in distribution of neutral red nor in location of marker enzymes. Per-

oxidase and a variety of acid hydrolases were distributed in the gradients at a mean density of approximately 1.23, which correlates well with the data of others (*10*). Most of the acid and alkaline phosphatase activities, measured with *p*-nitrophenyl phosphate as substrate (*11*), were considerably closer to the top of the gradients. This seeming discrepancy in distribution of acid phosphatase and (other) lysosomal hydrolases has been noted (*8*) with rabbit heterophile leukocytes and related to the presence of two acid phosphatases in these cells. In man, as in the rabbit, alkaline phosphatase of the leukocyte does not appear to be compartmentalized with the acid hydrolases and peroxidase.

In cystinotic leukocytes a large percentage of cystine migrated in the gradients with an average bouyant density of 1.23, along with the dense lysosomal particles. Cystinotic cells show this characteristic cystine peak after labeling with brief exposure to cysteine-^{35}S and also by the quantitative colorimetric technique, which is independent of tracer methodology and hence not influenced by rates of incorporation of exogenously supplied cysteine into intracellular cystine pools (Figs. 1 and 2). This cystine peak deep in the gradient was not seen in preparations of normal cells. In preparations of both normal and cystinotic cells, some cystine was always seen at the top of the gradients; this presumably represents noncompartmentalized cystine which had in part preexisted in these cells as the normal constituent cysteine, and possibly some cystine which had leaked out of intracellular organelles during preparation.

The small numbers of leukocytes available precluded identification of the mitochondrial band by enzyme assay in our gradients. However, mitochondria of human neutrophiles have a bouyant density of approximately 1.17 (*10*), a value that corresponds to that of tube 10 in our gradients where only minute amounts of cystine were detected.

The specific gravity of cystine crystals is approximately 1.73 (*12*). The fact that the cystine peak characteristic of cystinotic leukocyte gradients is found at the very much lower specific gravity of 1.23 indicates that the cystine occupying the lysosomes in leukocytes is probably not in the crystalline form in which it has been visualized in cystinotic reticuloendothelial cells from lymph nodes and in conjunctival macrophages. Cystine, predominantly in noncrystalline form, is apparently compartmentalized within a dense class of lysosomes in cystinotic leukocytes.

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Intestinal Calcium Absorption: Nature of Defect in Chronic Renal Disease

Abstract. *When compared to that of normal animals, calcium-binding protein activity of duodenal mucosa obtained from uremic rats was decreased. There was no change in this activity after vitamin D_3 therapy. In contrast, prior treatment with 25-hydroxycholecalcijerol resulted in increased transport of calcium-45 and calcium-binding protein activity in the intestine.*

The intestinal absorption of calcium is characteristically depressed in patients with chronic renal insufficiency (*1*) and in rats with experimentally induced chronic uremia (*2*). We have presented evidence for an abnormal metabolism of vitamin D_3 in subjects with chronic uremia and a decrease in concentration of a biologically active vitamin D_3 metabolite in the plasma (*3*). This metabolite, which is normally more potent than vitamin D_3 in effecting the intestinal transport of calcium, has been subsequently identified by De Luca and co-workers as 25-hydroxycholecalciferol (*4*). In our earlier studies we suggested that the derangements of vitamin D_3 metabolism noted in uremic subjects could account, at least in part, for the intestinal malaborption of calcium of chronic renal disease and the reported "resistance" of renal osteodystrophy to vitamin D therapy (*3*). Since there is mounting evidence that some of the effects of vitamin D or metabolites thereof on intestinal calcium transport may be mediated by the induction of a specific calcium-binding protein (Ca BP) (*5*), the possibility existed that, as a consequence of abnormal vitamin D metabolism, the activity of Ca BP was depressed in the chronic uremic state.

We now report that the concentration of Ca BP is decreased in the intestinal mucosa of rats with experimentally induced chronic uremia and defective intestinal calcium transport. Whereas concomitant increments in calcium transport and amounts of intestinal duodenal Ca BP were observed in normal rats after vitamin D_3 therapy, vitamin D_3 was ineffectual in reversing both the abnormal calcium transport and low concentrations of Ca BP in the mucosa of uremic rats. In contrast, 25-hydroxycholecalciferol was extremely effective in reversing the abnormalities in calcium transport and Ca BP activity.

The chronic uremic state was experimentally induced in male Sprague-Dawley rats (100 g) by unilateral (right) nephrectomy and ligation of most of the primary and secondary divisions of the main left renal artery. Sixty days after nephrectomy and segmental infarction of the contralateral kidney, when urea nitrogen in the blood was 70 mg per 100 ml or greater, the animals were placed on a low calcium diet for 4 days and then killed by decapitation. At death, the uremic animals were randomly distributed into one of two groups. In one group a