Annu. Rev. Nutr. 1989. 9:39–61

LYSOSOMAL MEMBRANE TRANSPORT IN CELLULAR NUTRITION[1]

William A. Gahl

Human Genetics Branch, National Institute of Child Health and Human Development, National Institutes of Health, Building 10, 9000 Rockville Pike, Bethesda, Maryland 20892

CONTENTS

INTRODUCTION

The lysosome has classically been pictured as an acidic bag of hydrolytic enzymes serving to degrade macromolecules. The manner in which a lyso-

[1]The US Government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

somal enzyme fulfills its life's function has long fascinated cell biologists. In cultured fibroblasts, synthesis of a lysosomal enzyme in the endoplasmic reticulum and modification in the Golgi apparatus are followed by targeting the enzyme to the lysosome by means of a mannose-6-phosphate recognition marker (54, 93). Lysosomal uptake and processing of the enzyme occurs before it becomes active in the acidic lysosomal milieu. While these aspects of lysosomal function have been elegantly elucidated (79), the fate of the products of lysosomal catabolism has been largely ignored. Yet the amino acids, monosaccharides, and lipids produced in the lysosome also serve as precursors to macromolecules awaiting synthesis in the cytosol. To participate in their recycling, small molecules must cross the lysosomal membrane, often by means of a specific transport system. This process of salvaging the cell's "building blocks" is a primary nutritional function of lysosomes.

Another role of the lysosome in cellular nutrition merits mention. The lysosome serves as a conduit or, more descriptively, a gate-keeper for the movement of certain nutrients into and out of cells. The respective processes involved are endocytosis and exocytosis, the former being mediated by specific, high-affinity receptors whose activity comes under regulatory control. As for lysosomal enzymes, uptake of the receptor-ligand complex into the lysosomes has been studied extensively, but the fate of the small ligand, after being displaced from its receptor within the lysosome, has not been emphasized. Yet movement of this small ligand across the lysosomal membrane and into the cytosol is essential for the maintenance of cellular nutrition.

This review focuses on the movement of cellular nutrients, present in lysosomes by virtue of either enzymatic degradation of macromolecules or of receptor-mediated endocytosis, into and out of lysosomes. The processes of lysosomal hydrolysis of macromolecules and receptor-mediated endocytosis are discussed using a few well-defined systems for illustration. Both recognized and potential lysosomal transport disorders are also mentioned.

SMALL MOLECULES PRODUCED BY LYSOSOMAL HYDROLYSIS

The lysosome contains enzyme systems capable of hydrolyzing proteins, glycosaminoglycans, and nucleic acids. As noted in 1969 by Lucy (70), the products of this catabolism, including amino acids, monosaccharides, and nucleosides, "presumably cross the membrane in the outward direction subsequent to the degradation by hydrolytic enzymes of the corresponding, engulfed, polymeric materials."

Amino Acids

Intracellular proteins are degraded by at least two nonlysosomal cytoplasmic systems, including one with a neutral pH optimum and a requirement for

adenosine triphosphate (ATP) (31) and another involving short-lived proteins bound to ubiquitin (58). But under nutritionally depleted conditions, some intracellular proteins, as well as exogenous proteins that enter the cell by pinocytosis, are degraded by lysosomal proteolysis (58). This proteolysis is achieved by a variety of lysosomal proteinases (47). By the early 1970s, these proteinases were recognized to consist of peptidases and amidases (8) and to include leucine aminopeptidase, an aminodipeptidase, an aminotripeptidase, a carboxypeptidase (cathepsin A), an iminopeptidase, as well as prolidase, cathepsin D, elastase, and collagenase (6). Currently, at least six lysosomal proteinases are known to exist. They are divided into cysteine proteinases (cathepsins B, H, L, and S) and aspartic proteinases (cathepsins D and E) (65). The cysteine proteinases have been purified from several different species and their activities compared (75); their processing and targeting to the lysosomes has also been examined (80, 81).

In addition to lysosomal proteases, the proteins to be degraded must themselves arrive within the lysosomes. Since lysosomal proteolysis of endogenous proteins in human fibroblasts requires new protein synthesis, the existence of a ubiquitinlike molecule that targets proteins for lysosomal degradation has been proposed (I 10).

The study of lysosomal proteolysis has been advanced by the investigation of the accumulation of a particular amino acid, cystine, within lysosomes of fibroblasts from patients with the lysosomal storage disease cystinosis. (See below for a description of this disease.) In these cells, lysosomal cystine accumulation is a measure of lysosomal proteolysis and is inhibited by chloroquine and ammonium chloride (112), agents that alkalinize lysosomes and inhibit acid hydrolases. Several studies have demonstrated that various intracellular proteins act as sources of cystine for the mutant cystinotic cells (108, 109, 112). Not surprisingly, cystine was the first amino acid for which a lysosomal membrane carrier was described, and cystinosis the first disorder reported to be caused by impaired lysosomal transport.

LYSOSOMAL CYSTINE TRANSPORT Cystine produced by lysosomal protein hydrolysis needs to traverse the lysosomal membrane and enter the cytosol, where 5–10 mM glutathione reduces it to its free thiol, cysteine. That transport process is carrier-mediated and bidirectional, as evidenced by saturability of cystine egress in polymorphonuclear leukocyte lysosomes (35) and by the demonstration of cystine counter-transport (39). This phenomenon, in which the rate of uptake of a tracer amount of a radioactive substance is stimulated by saturating concentrations of the nonradioactive substance on the other side of the membrane, proves the existence of a carrier within the membrane. In the case of cystine, lysosomes were loaded with nonradioactive cystine by exposure to cystine dimethylester (44, 91), which is rapidly hydrolyzed to free cystine within lysosomes (103). Uptake of 8 μM

[^{3}H]cystine into these lysosomes over a given time period was compared with [^{3}H]cystine uptake into nonloaded lysosomes. A difference between the two rates represented counter-transport, and this difference was readily apparent in normal leukocyte lysosomes (39).

By using the technique of counter-transport, lysosomal cystine transport was found to be temperature dependent, with a Q_{10} of approximately 2.0 and an energy of activation of 11.4 kcal/mol (39). The transport is specific for the L-isomer of cystine and has strict structural requirements for ligand binding. The ligand must contain two amino groups separated by six (not eight) methylenes or sulfurs. No other amino acid except cystathionine is known to be recognized by the lysosomal cystine carrier, whose apparent K_m for cystine approximates 5×10^{-4} M. Whether or not the lysosomal cystine carrier requires energy for its operation has not been absolutely determined. Experiments involving the addition of exogenous ATP (62, 63, 101) are difficult to interpret because, besides its energy-supplying effects, ATP also stimulates lysosomal proton pump activity. Lysosomal acidification itself may play a role in stimulating cystine transport. Our current understanding is that ATP has no effect on cystine transport out of highly loaded polymorphonuclear leukocyte lysosomes but does stimulate cystine transport out of less well loaded leukocyte lysosomes (48).

Patients with cystinosis lack sufficient cystine transport to remove cystine from the lysosomes. This defect has been demonstrated by egress experiments in leukocytes (35, 40), lymphoblasts (63), and fibroblasts (89) and by counter-transport experiments in polymorphonuclear leukocytes (39). Heterozygotes for cystinosis have half the maximal velocity of lysosomal cystine egress (35) and counter-transport (36), which verifies the gene dosage effect in this autosomal recessive disease. As a consequence of impaired transport of cystine, the disulfide accumulates within lysosomes and forms crystals in many tissues. Cellular destruction leads to renal Fanconi syndrome in the first year of life, glomerular failure by approximately 10 years of age, growth failure, thyroid deficiency, and impairment of other essential organ functions (34, 38). Corneal crystal formation contributes to photophobia and recurrent corneal erosions. All these clinical manifestations appear due to cystine accumulation, since cytoplasmic cysteine levels appear normal in cystinotic cells (100). Thus, although in some cases lysosomal proteolysis may contribute to cellular nutrition by provision of amino acids, this source does not appear to be essential in the case of cysteine.

LYSOSOMAL LYSINE TRANSPORT After the cystine carrier was described, several other amino acid transport systems were discovered. The first was a system resembling the plasma membrane system y^+ and recognizing the cationic amino acids arginine, lysine, ornithine, and histidine, as well as

diaminobutyrate and 2-aminoethyl cysteine (89). *Trans*-stimulation experiments revealed substantial ligand specificity, stereospecificity for the L-isomer of lysine, inhibition of transport by the lysosomal alkalinizing agent chloroquine, and lack of sodium dependence. The Q_{10} is 2.2 and the energy of activation is 17 kcal/mol.

Later studies demonstrated important differences between the lysosomal transport system redesignated system c for "cationic" and the plasma membrane system y^+ (90). These differences include pH-dependent arginine influx in the lysosomal but not the plasma membrane system, an 8-fold higher apparent K_m for arginine exhibited by the lysosomal system, and different amino acid analog specificities for the two transport systems.

Perhaps the most important aspect of the lysosomal system c is its ability to recognize the mixed disulfide cysteine-cysteamine (89), a structural analog of lysine itself. Cysteamine is a known cystine-depleting agent (111), whose amine group confers the ability to freely traverse the plasma and lysosomal membranes and whose free thiol allows it to participate in a disulfide interchange reaction with cystine. The products are cysteine and the mixed disulfide cysteine-cysteamine. That cysteine could readily exit cystinotic lysosomes was known (40), and that the mixed disulfide cysteine-cysteamine could also circumvent the transport defect in cystinosis now became apparent (41); the mixed disulfide did so via the lysine carrier, which is not impaired in cystinotic lysosomes (89). This mechanism of action explains cysteamine's remarkable efficiency in depleting cystinotic cells of cystine (111), and the striking clinical benefits it provides vis-à-vis growth and preservation of renal function (37) and corneal crystal dissolution (64).

LYSOSOMAL TYROSINE TRANSPORT The technique of counter-transport was employed to characterize a transport system for tyrosine and neutral amino acids in the lysosomal membrane of rat thyroid (FRTL-5) cells (10). These cells incorporate iodine into thyroglobulin, synthesize thyroxine (T_4), triiodothyronine (T_3), diiodotyrosine (DIT), and monoiodotyrosine (MIT), and respond to thyroid-stimulating hormone (TSH) by increasing their thyroxine-producing functions. The lysosomal carrier, which resembles the plasma membrane L system, recognizes tyrosine, leucine, histidine, phenylalanine, and tryptophan but not cystine or lysine. It demonstrates stereospecificity but not cation or acid pH dependence. The apparent K_m for tyrosine is approximately 100 μM and the energy of activation 9.7 kcal/mol (10). The system was stimulated approximately 7-fold by TSH (53).

Lysosomal tyrosine transport may be critical for thyroid cells because the tyrosine residues are reused for synthesis of thyroglobulin in the endoplasmic reticulum. But even more vital should be the salvage of iodine, since dietary iodine intake is limited. When thyroglobulin is hydrolyzed to amino acids in

the lysosome, its iodine-containing moieties include T_4, T_3, DIT, and MIT. The T_4 and T_3 are functional and must be delivered to the circulation for binding to thyroid-binding globulin or thyroid-binding prealbumin. But DIT and MIT must recirculate their iodine within the cytoplasm and, to achieve this goal, must traverse the lysosomal membrane. A transport system performing this function has recently been described in FRTL-5 cells by the measurement of egress, uptake, and counter-transport of [^{125}I]MIT (4). The system exhibits an apparent K_m for MIT of approximately 2 μM and probably recognizes tyrosine and DIT as well as MIT.

Preliminary evidence suggests that the tyrosine and MIT transport systems may be one and the same (H. Andersson, personal communication). But whether or not this suggestion proves correct, the MIT carrier appears to play a crucial and specialized role, i.e. iodine salvage, in the nutrition of a highly differentiated cell, the thyroid cell.

OTHER LYSOSOMAL AMINO ACID TRANSPORT SYSTEMS In 1987, human fibroblasts were employed to demonstrate lysosomal transport systems for several other amino acids, including proline, serine, threonine, and alanine (87). Proline traverses the lysosomal membrane by two systems, with apparent K_m values of 0.01 and 0.07 mM, respectively. The low-affinity system p has high specificity for proline and 3,4-dehydro-L-proline. The high-affinity system recognizes unbranched neutral amino acids including sarcosine and *N*-methyl-L-alanine, as well as proline. This system is similar in some respects to system A present in plasma membranes. The pH optimum for lysosomal proline uptake is 6.4, and extralysosomal MgATP does not influence lysosomal proline uptake.

Alanine, serine, and threonine are transported to a small degree by the high-affinity proline system f but largely by a separate carrier system e (87). The lysosomal system e resembles the plasma membrane system ASC, which carries alanine, serine, cysteine, and similar amino acids, including threonine.

Perhaps the only amino acid transport system left to be characterized in the lysosomal membrane would carry dicarboxylic amino acids, and hints that this system exists have already been published (87). Lysosomal amino acid transport systems have been reviewed recently (22a).

Carbohydrates

Lysosomes contain a cadre of enzymes that hydrolyze mucopolysaccharides to their constituent monosaccharides. The absence of specific hydrolases leads to well-defined lysosomal storage diseases known as the mucopolysaccharidoses (72). The presence of the enzymes means that the lysosome must have some means of ridding itself of osmotically active simple sugars. In at

least some cases, this appears to be accomplished through carrier-mediated transport.

SIALIC ACID Sialic acid, or *N*-acetylneuraminic acid, is synthesized in the cytosol from glucosamine through a series of intermediates, including *N*-acetylmannosamine (38). Sialic acid reacts with cytidine triphosphate (CTP) to form cytidine monophosphate (CMP)-sialic acid, which enters the trans-Golgi, where a sialyltransferase adds sialic acid to a growing carbohydrate chain. The resulting glycoconjugate finds its way to the lysosome, where neuraminidase activity removes a terminal sialic acid. Subsequent metabolism and reutilization of free sialic acid occurs outside the lysosome, which suggests that free sialic acid must traverse the lysosomal membrane to participate in further reactions.

This process of lysosomal sialic acid transport was investigated in cultured human fibroblasts that had been loaded with free sialic acid by exposure to between 30 and 100 mM *N*-acetylmannosamine for several days (113). The lysosomal free sialic acid levels achieved, 90 picomoles per unit of hexosaminidase activity, allowed measurement of free sialic acid egress from lysosomes isolated from the normal, loaded fibroblasts. Initial velocity of sialic acid egress increased linearly with loading and was temperature dependent, with a Q_{10} of approximately 2.3 (94).

As for lysosomal cystine transport, interest in free sialic acid transport was engendered by a human disease in which the small molecule accumulates within lysosomes. Salla disease is an autosomal recessively inherited Finnish disorder, in which patients are normal at birth but develop truncal ataxia by one year of age (38). Variable degrees of psychomotor retardation occur, and longevity is slightly reduced. A much more severe variety of the general disorder, infantile free sialic acid storage disease (ISSD), is fatal in infancy or childhood (38).

Cells from both Salla disease and ISSD patients display engorged lysosomes and store 10- to 200-fold normal amounts of free sialic acid. Transport of the negatively charged sugar across the lysosomal membrane is negligible in Salla disease (94) and ISSD (114). Although saturation kinetics were not demonstrated for normal lysosomal sialic acid transport (94), the very existence of mutants defective in this function strongly suggests that a specific carrier exists for transporting free sialic acid out of lysosomes.

OTHER SUGARS The ability of sugars to cross lysosomal membranes has been studied indirectly. In 1969, Ehrenreich & Cohn demonstrated that feeding of certain dipeptides and tripeptides to cultured mouse macrophages induced vacuolation (30), presumably because the inability of these molecules to exit the lysosome after pinocytosis resulted in osmotic engorgement of the

lysosomes. They later concluded that, although the shape and charge of small molecules influenced the ability of these molecules to traverse the lysosomal membrane, in general, compounds of molecular weight less than 230 could exit the lysosome freely by diffusion, while large substances could not. As reported above, however, several amino acids of molecular weight less than 200 do appear to employ carriers for transport across the lysosomal membrane.

The early studies on lysosomal permeability were extended by Lloyd (69), who measured lysosomal enzyme latencies after preincubation with various disaccharides. The latencies did not decrease, because the lysosomal membrane was impermeable to these sugars. In contrast, monosaccharides, including D-glucose, D-mannose, D-arabinose, D-galactose, D-xylose, and D-ribose, penetrate the lysosomal membrane rapidly enough to decrease lysosomal enzyme latency, i.e. to osmotically damage the integrity of the lysosomal membrane.

The osmotic protection method was further exploited to make a case for the facilitated diffusion of sugars across the lysosomal membrane. Uptake of monosaccharides by rat liver lysosomes occurred in increasing order: 2-deoxy-D-glucose, D-mannose, D-galactose, D-ribose, and 2-deoxy-D-ribose (28), although whether D-glucose and D-ribose share a common carrier has lately been contested (11). Based on demonstrations of temperature dependence (Q_{10} approximately 2.8), stereospecificity, inhibition by phlorizin and cytochalasin B, and competition among sugars, sugar uptake into rat liver lysosomes was concluded to be a facilitated process. Since membrane transport is bidirectional if conditions are set properly, the sugars tested can presumably exit as well as enter lysosomes.

In subsequent work, the direct uptake of radioactive sugars into rat liver lysosomes was measured, and the previous results obtained by the osmotic protection method were confirmed. In addition, saturation kinetics were demonstrated, and a K_m for glucose uptake of 48 ± 18 mM was determined (73).

N-ACETYLHEXOSAMINES The lysosomal degradation of glycoproteins and glycosaminoglycans produces *N*-acetylglucosamine and *N*-acetylgalactosamine (72). Since radioactive experiments revealed that the total amount of these compounds appearing outside the lysosome of cultured human fibroblasts corresponded to the amount released by degradation, Rome & Hill (95) concluded that the egress of these *N*-acetylhexosamines from the lysosome must be very rapid. But even more significant is the finding that both *N*-acetylglucosamine and *N*-acetylgalactosamine were extensively reused by the cell, which underscores the importance of lysosomal membrane transport of these small molecules in cellular nutrition.

Nucleosides

Lysosomal ribonucleases produce nucleic acids that the lysosome must eliminate. To study this process, Percoll-purified lysosomes were isolated from human fibroblasts and exposed to tritiated adenosine (88). The uptake of this nucleoside was temperature sensitive (Q_{10} 2.0; energy of activation 12.9 kcal/mol), saturable (K_m approximately 8 mM at pH 7.0 and 37°C), and inhibited by other nucleosides, including 2'-deoxyadenosine, inosine, cytidine, uridine, and thymidine, but not by D-ribose, L-phenylalanine, or L-leucine. The transport of nucleosides from lysosome to cytosol would provide a means for the salvage of these crucial compounds for reuse by the cell.

SMALL MOLECULES PRESENT IN THE LYSOSOME BECAUSE OF RECEPTOR–MEDIATED ENDOCYTOSIS

The uptake of several small molecules is mediated by specific, high-affinity receptors on the plasma membrane surface. In general, the receptor-ligand complexes are internalized to endosomes where the acidic environment cleaves off the ligand. The small molecules that enter the cell by this process either proceed to lysosomes, from which they must eventually exit, or exit directly from endosomes. The receptor returns to the plasma membrane surface for recycling.

The entire process of receptor-mediated endocytosis is perhaps best exemplified by the uptake of cholesterol present within low density lipoproteins (LDL).

Cholesterol

Cholesterol is a precursor of steroid hormones and a component of membranes essential for virtually all cells of the body. The intestine absorbs exogenous cholesterol and the liver produces endogenous cholesterol, but both these sources of cholesterol must have a delivery system to be useful to peripheral tissues. Cholesterol itself is insoluble in aqueous solutions, and cholesterol esterified to long-chain fatty acids is too hydrophobic to traverse plasma membranes and enter distant cells. So, lipoproteins and their receptors are necessary to achieve, among other things, cholesterol transport (16).

Low density lipoproteins are spherical particles of 3×10^6 daltons composed of a core of 1500 cholesteryl ester molecules surrounded by a coat of phospholipid, unesterified cholesterol and a specific protein, apoprotein B-100 (16). Within the circulation, LDL particles are derived from intermediate density lipoproteins (IDL) by removal of triglycerides; during the process, all apoprotein E is also removed from the IDL. The LDL and IDL particles are specifically recognized by LDL receptors by virtue of their apo B-100 and apo E content, respectively. The LDL receptors are located on the surfaces of

cholesterol-seeking cells, clustered in coated pits. Studied in cultured fibroblasts (2, 3), these pits were found to pinch off to form endocytic vesicles whose cytoplasmic coat consists largely of a protein called clathrin (83). The clathrin-coated endocytic vesicles fuse to become endosomes and undergo a drop in pH due to the activity of an ATP-driven proton pump (74, 76). Their acidity causes dissociation of LDL from its receptor. The LDL is delivered to lysosomes by fusion with the endosome and is degraded by acid hydrolases. The products of this hydrolysis, including cholesterol and amino acids, must enter the cytoplasm to contribute to cellular nutrition. The LDL receptors themselves cluster in the endosomal membrane, pinch off to form a recycling vesicle, and recirculate to the plasma membrane (16). The complete cycle from lysosome to plasma membrane takes approximately 10 minutes (45).

The process of cholesterol homeostasis operates via several control mechanisms. First, feedback inhibition is exerted on 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoA reductase), the rate-limiting enzyme in cholesterol synthesis, by LDL-derived cholesterol, through suppression of transcription (71) and increased enzyme protein degradation (42). Second, exogenous cholesterol activates acyl CoA: cholesterol acyltransferase (ACAT), which esterifies cholesterol for storage in the cytoplasm (92). Finally, LDL-derived cholesterol acts on LDL receptor mRNA to reduce the number of LDL receptors synthesized (15).

A good deal is known about the LDL receptor (16). It has five functional domains: one for ligand binding; one with epidermal growth factor precursor homology; one with only sugars attached; a membrane spanning region; and a cytoplasmic domain. Mutations in the various domains result in the human disease familial hypercholesterolemia, characterized by early atherosclerosis and myocardial infarction (46). The role of the lysosome in LDL receptor turnover also is clear: Its acidic environment dissociates LDL from its receptor and allows recirculation of the receptor.

But what happens to the products of lysosomal degradation of LDL? The amino acids can be transported out of the lysosome by membrane carrier systems described above. The cholesterol esters are hydrolyzed to cholesterol and free fatty acids by an acid lipase, whose deficiency in Wolman's disease causes a fatal lysosomal storage of cholesteryl esters (5).

What does the lysosome do with the newly produced cholesterol? One possibility is that the lipophilic cholesterol molecules spontaneously partition within the lysosomal membrane. Cholesterol distributes unevenly within membranes according to the membrane's composition in general and phospholipid content in particular (121). Of course, cholesterol would then have to get out of the lysosomal membrane to be reesterified (for storage) by ACAT, an enzyme in the rough endoplasmic reticulum (92). Moreover, the

spontaneous partitioning of cholesterol into a membrane appears too slow to account for the observed rate of movement of cholesterol (26).

A second possibility arises from the description of cholesterol binding proteins or transfer factors. In 1975, cholesterol was reported to exchange between rat liver microsomes, mitochondria, and erythrocyte plasma membranes in vitro (9). This exchange was enhanced by a soluble fraction component termed a *cholesterol exchange protein*. Later, a sterol carrier protein of molecular weight 47,000 (SCP_1) was purified 575-fold from rat liver cytosol and found to serve as a carrier for squalene in its enzymatic conversion to lanosterol by rat liver microsomal membranes (102). A different sterol carrier protein (SCP_2) of molecular weight 13,500 was also purified from rat liver cytosol (82). SCP_2 is required for cholesterol transfer from cytoplasmic lipid inclusions to the mitochondria and from the outer to the inner mitochondrial membrane in adrenal cells (99). Sterol carrier proteins are proposed to act by intermembrane transfer of sterol substrate rather than by a classic substrate carrier mechanism (17). A fatty acid binding protein, distinct from the sterol carrier proteins, also exists (99). Cholesterol and fatty acids appear to be transferred from lysosomal membranes by binding proteins identical or analogous to those already described for other intracellular lipid transport functions.

The investigation of cholesterol movement across lysosomal membranes may be aided by study of an unsolved inborn error of metabolism, Niemann-Pick Type C disease (13). The name of the disease derives from what will probably prove to be a secondary or incidental deficiency of sphingomyelinase activity. More related to the basic defect is that fibroblasts from these patients fail to esterify LDL-derived cholesterol (85), despite normal ACAT activity. LDL uptake and internalization are normal (86). Normal cholesterol ester lipase activity, combined with evidence of impaired cholesterol efflux (77), cholesterol accumulation in perinuclear inclusions (66), and cholesterol storage in subcellular fractions containing lysosomal enzymes (84), suggest a lysosomal locus for storage of free cholesterol. The cellular consequence of this defect is that LDL-derived cholesterol fails to suppress endogenous cholesterol synthesis and LDL receptor-mediated uptake (68). But with the solution to the defect in Niemann-Pick Type-C disease may well come an understanding of how LDL-derived cholesterol normally traverses the lysosomal membrane to fulfill its metabolic functions.

Iron

All cells require iron for certain catalytic steps that rely upon the metal's ability to change its redox state. Iron fulfills its crucial role in cellular nutrition after crossing the plasma membrane by receptor-mediated endocyto-

sis. It enters cells bound to the transport protein transferrin and accumulates in cells bound to the storage protein ferritin. Although lysosomes per se may not be involved in iron uptake into cells, the endocytic vesicle, an acidic compartment considered prelysosomal in some systems, plays an integral role.

Transferrin is a single chain glycoprotein of molecular weight 76,000–80,000 with two iron binding sites and no sulfhydryl groups (1). It contains 6% carbohydrate, linked to asparagine residues 413 and 611 and terminating in sialic acid (52). Transferrin has two globular domains and binds ferric iron with the concomitant binding of a bicarbonate or carbonate ion at pH 7.4. Serum transferrin, containing iron, attaches to cell surface receptors that are saturable, specific, and reversible in their binding (1, 52, 61). The transferrin receptor has been studied on reticulocyte, hepatocyte, placental, macrophage, and lymphocyte membranes in a variety of species, including man. The receptor is a disulfide of two identical 90,000–95,000 dalton subunits and contains three oligosaccharide chains *N*-linked to asparagine (52, 115). A large portion of the transferrin receptor is exposed to the cell surface to make it available for binding of transferrin; trypsin treatment releases a 70,000-dalton fragment of the receptor. The association constant for the receptor-transferrin complex approximates 2–7 $\times$ 10^{-9}M (115).

The transferrin-receptor complex is taken up by invagination of clathrin-coated pits. As endosomes form, the vesicles become acidic, which causes dissociation of iron from transferrin (52). The receptor-transferrin complex returns to the cell surface, where the neutral pH allows dissociation of the two components, each intact for reuse. In this fashion, the transferrin receptor recycles repeatedly, delivering transferrin-bound iron to the inside of the cell.

That iron accumulates intracellularly. It is bound to ferritin, a 450,000–molecular weight spherical protein with a central cavity (1). Each ferritin molecule, which consists of 24 subunits, binds 2500 iron atoms in its central core. The uptake and release of iron by ferritin are not well-defined processes, but ferritin synthesis clearly increases in response to iron exposure (78). This increase in ferritin, along with an iron-induced decrease in transferrin receptor synthesis, serves to protect the cell against iron toxicity. When excess iron threatens a cell, the transferrin receptor transports less iron in, and ferritin binds and detoxifies the extra iron that does get in.

The regulation of the iron content of the cell occurs through posttranscriptional mechanisms. There exists in the 5' nontranslated leader region of human ferritin H-chain mRNA a *cis*-acting iron-responsive element (56, 57). This conserved, stem-loop structure regulates translation of ferritin mRNA so that excess iron stimulates ferritin synthesis. Five similar iron-responsive elements, also stem-loop structures, have been identified in the 3' untranslated region of the transferrin receptor's mRNA (20). These RNA sequences regulate the production of the transferrin receptor by altering the level

of receptor mRNA (19, 20). In the presence of excess iron, a putative regulatory molecule would be bound by iron and, therefore, be unavailable for binding to the iron-responsive element of the receptor's mRNA. As a consequence, the mRNA would be degraded (20).

Certain questions remain in this scheme of iron metabolism and its regulation. How iron leaves the endosome to bind to ferritin has not been determined; presumably some transport across the vesicular membrane is required. If this transport step were impaired, iron would be expected to accumulate within endosomes rather than within ferritin. We know of no such disorder.

Nor has it been determined what causes the most common aberration of iron homeostasis, hemochromatosis (12). This autosomal recessive disorder results in vastly increased amounts of iron, stored as insoluble aggregates known as hemosiderin. Clinical symptoms include those of a cardiomyopathy and parenchymal liver disease. Basic research into the normal disposition of cellular iron, using hemochromatosis as a model mutation, may also shed light on the cellular and endosomal handling of other, related metals.

Vitamin B_{12}

The two primary functions of vitamin B_{12}, or cobalamin (Cbl), involve the mitochondrial conversion of methylmalonyl CoA to succinyl CoA using 5'-deoxyadenosylcobalamin as coenzyme and the methylation within the cytoplasm of homocysteine to methionine using methylcobalamin as coenzyme and 5'-methyltetrahydrofolate as the immediate methyl donor (23). Cobalamin enters the cell bound to transcobalamin-II (TC-II); a specific cell surface receptor (32, 33) binds the TC-II–Cbl complex. Studies in cultured human fibroblasts (122) and human hepatoma cells (51) have shown that the TC-II–Cbl complex is internalized via endocytosis, with subsequent formation of a secondary lysosome. There, the acidic environment degrades the TC-II to its constituent amino acids, and cobalamin is released into the cytoplasm to bind to its apoenzymes. There does not appear to be feedback regulation of TC-II–Cbl receptor synthesis by intracellular cobalamin, such as is found in cholesterol homeostasis (122).

The importance of the movement of free cobalamin out of lysosomes and into the cytoplasm is emphasized by an apparent defect in this function in two patients with methylmalonic aciduria, one with developmental delay and the other with homocystinuria (96, 97, 116a). The original patient is developing normally with cyanocobalamin therapy (23). Her cultured fibroblasts and Epstein-Barr-virus-transformed lymphocytes accumulate free cobalamin in their lysosomes because of an apparent inability to transport cobalamin to the cytoplasm of the cell (96). Endocytosis of the TC-II–Cbl complex and release of cobalamin from TC-II are normal. Fibroblast studies (97) showed a func-

tional impairment of the major cobalamin-dependent methylation enzymes in this disorder, which has been termed the *cobalamin F mutation*.

OTHER POTENTIAL LYSOSOMAL TRANSPORT SYSTEMS AND POSSIBLE DEFECTS

In addition to the fairly well characterized systems mentioned above, there are several small molecules whose intracellular routing and metabolism have not been fully elucidated. These molecules are potential candidates for compounds that require transport across the lysosomal membrane to participate in further cellular metabolism.

Copper

In the adult in steady state, approximately 2 mg of copper is absorbed in the intestine and excreted in the bile each day (25, 118). Absorbed copper enters the plasma, where it is loosely bound to albumin or amino acids and transported to the liver. There, it can be incorporated into ceruloplasmin, a glycoprotein that binds more than 90% of the copper in human plasma, or into other copper-containing enzymes such as cytochrome oxidase, superoxide dismutase, tyrosinase, dopamine β-hydroxylase, and lysyl oxidase (25, 104). Alternatively, copper can be bound tightly to metallothionein, a 6,000 to 10,000-dalton protein containing 30% cysteine residues, or it can be taken up by lysosomes prior to excretion into biliary canaliculi.

There are three primary diseases of copper metabolism. The most common is Wilson's disease, an autosomal recessive disorder of copper excess with hepatic, neurologic, and combined hepatic-neurologic symptomatology occurring with equal frequency (25). The hepatic disease is clinically nonspecific but can be acute and fatal. The neurologic disease occurs because of basal ganglia involvement and manifests as dysarthria, poor coordination, and involuntary movement. Central nervous system involvement, as well as Kayser-Fleischer rings in the cornea, osteoporosis, and renal tubular Fanconi syndrome, all result from copper overflow from the liver. Treatment is lifelong chelation therapy with D-penicillamine. The basic defect in Wilson's disease must be related to the inability of affected individuals to incorporate copper into ceruloplasmin and to excrete copper in the bile. How these two processes are related remains unknown, but the role of the lysosome in hepatic copper excretion is becoming increasingly accepted. In 1966, de Duve & Wattiaux (27) suggested that "extrusion in bile is the major pathway of unloading of the lysosomes of hepatic parenchymal cells." Later, this hypothesis was bolstered by the demonstration of coordinate secretion of lysosomal enzymes into the bile of rats (67). The hepatic exocytosis of lysosomal contents apparently includes copper, since tracer studies showed an identical

specific radioactivity of ^{64}Cu in the bile compared with the hepatic lysosome in a Wilson's disease patient who underwent cannulation and biopsy after receiving an injection of the radiolabeled copper (105).

All this evidence points to a defect in the ability of hepatic lysosomes to either take up copper or to subsequently disgorge their contents, including copper, into the bile. The latter defect would be expected to cause problems more global than those of excess copper accumulation. The former explanation is supported by the finding of excess copper in a cytoplasmic location early in Wilson's disease (43). The cytosolic copper is bound to metallothionein but may well be a secondary phenomenon, with another defect causing copper accumulation first. That defect may be impaired movement of copper, either free or bound to metallothionein, into the lysosome. Metallothionein binds copper extremely tightly (29), so this protein might need to be hydrolyzed to its constituent amino acids for the copper-metallothionein complex to release its copper. Although zinc-metallothionein can be degraded by lysosomal proteases in vitro, copper-metallothionein cannot (14, 55), a fact that suggests that some in vivo modification of the copper-metallothionein complex, such as a change in redox state, may be required prior to any putative degradation by lysosomes. Since cultured fibroblasts may manifest the copper storage defect in Wilson's disease (22), these cells might be used to further explore lysosomal handling of copper in the normal and diseased states.

A second abnormality of copper metabolism is Menkes' disease, an X-linked disorder due to cellular copper deficiency and, consequently, a lack of active copper-containing enzymes (25). Patients with Menkes' disease usually die of neurologic disease in infancy and have kinky, pale hair, osteoporosis, tortuous arteries, hypothermia, developmental delay, and seizures. Hepatic, brain, and serum copper are low, but duodenal and kidney copper levels are elevated. In general terms, the basic defect in Menkes' disease lies in defective cellular uptake of copper.

Electron microscopy studies of intestinal mucosal cells and cultured fibroblasts show a striking accumulation of copper on the plasma membrane surface of Menkes' disease, but not normal, cells (60). Although the mechanism of copper uptake by cells has not been elucidated, these findings in Menkes' disease invite an analogy between copper and iron in their mode of uptake. There may well be a binding protein for copper whose receptor is ingested by endocytosis in clathrin-coated pits, with intracellular release of copper being achieved by the acidification of the endosome; the receptor may then be recycled to the cell surface. In such a scheme, the defect in Menkes' disease would be in internalization or release of the tightly bound copper, and copper would accumulate on or near the cell surface. This copper would not be accessible for further metabolic interactions, and the end result would be

copper deficiency. The defect in Menkes' disease is manifest in cultured fibroblasts (18), a system that should provide a fertile field for future studies.

The third important disorder of copper is Indian childhood cirrhosis, a severe disease characterized by hepatic cirrhosis and death in early childhood (106). Hepatic copper, which accumulates to levels higher than those in Wilson's disease, takes the form of electron-dense aggregates. These aggregates also contain large amounts of sulfur, which may represent the presence of metallothionein. The defect in Indian childhood cirrhosis remains unknown but is presumably related to failure of hepatic lysosomes to excrete copper, as in Wilson's disease. The formation of intracellular copper-containing aggregates in Indian childhood cirrhosis and the ability to produce normal levels of ceruloplasmin differentiate the two diseases pathologically.

Tyrosine and Albinism

There are at least ten different types of autosomally inherited oculocutaneous albinism and several varieties of X-linked and autosomal-recessive ocular albinism (119). These disorders result from defects in the synthesis of melanin, a pigment formed by spontaneous autooxidation of dopaquinone. Dopaquinone is produced by oxidation of dopamine, which is made by hydroxylation of tyrosine; the enzyme tyrosinase catalyzes both these reactions.

The synthesis of melanin occurs within melanosomes, which are the melanocyte's counterpart of lysosomes. Some albinos lack tyrosinase activity, as determined by assay of hair bulbs, which contain melanocytes. But the various causes of albinism in patients with normal tyrosinase activity remain mysteries (119). One possibility is that, under normal circumstances, tyrosine must be taken up into melanosomes against a concentration gradient for subsequent melanin synthesis; certain albinos might lack such a carrier-mediated transport system. As noted above, a tyrosine and neutral amino acid transport system has been described in the lysosomes of rat thyroid cells (10); whether such a system exists in the melanosomes of melanocytes and is defective in certain types of albinism might be determined using melanocytes in culture (116).

Sulfate

The degradation of sulfated glycosaminoglycans by lysosomal enzymes such as iduronate sulfatase and heparin *N*-sulfatase yields free sulfate within lysosomes (72). Although the lysosomal membrane has been considered permeable to neutral molecules of low molecular weight, charged species may require a specialized transport system to move out of lysosomes. In fact, when the permeability of rat lysosomal membranes to different anions was assessed by the osmotic lysis technique, sulfate was shown to traverse the lysosomal membrane very poorly (21). Conversely, in human fibroblasts the rate of [^{35}S]sulfate released from glycosaminoglycans paralleled that released from

lysosomes (95). Thus, lysosomal membrane transport appeared not to be rate limiting in the degradative pathway of sulfated glycosaminoglycan (lysosome) to free sulfate (cytosol). Nevertheless, a defect in lysosomal sulfate transport may well exist and take the clinical form of a lysosomal storage disorder in which all known lysosomal enzyme activities are normal.

Hexuronic Acids

The hexuronic acids, glucuronic acid and iduronic acid, are monosaccharides produced by the enzymatic degradation of glycosaminoglycans within the lysosome (72). As charged sugars, they might require a specific transport system to traverse the lysosomal membrane; alternatively, the sialic acid porter may also be involved in hexuronic acid transport. Again, a defect in a putative hexuronic acid transport system might present as an unknown lysosomal storage disorder, with normalcy at birth and progressive clinical deterioration beginning in the first year of life.

Vitamins

With cobalamin providing the precedent for a vitamin that requires movement out of the lysosome to incorporate into apoenzymes (96), one can look for other vitamins that might be similarly handled.

Biotin (59) serves as cofactor for several carboxylases and is produced by the cleavage of biocytin (ε-*N*-biotinyl-L-lysine) or biotinyl peptides by biotinidase (24). Biotinidase might be expected to be a lysosomal enzyme. Because its activity is not enriched in the lysosomal fraction, however, biotinidase is considered a secreted enzyme (120). Extracellular biotin itself, then, must be taken up across the plasma membrane. Such uptake in rat intestine has a component of facilitated transport (98), and a receptor for biotin has been described in mouse hepatic plasma membranes (117). Receptor-mediated endocytosis of biotin has not been conclusively demonstrated, but future studies may reveal that a biotin-protein receptor complex requires cleavage within an acidic endosome or lysosome, with subsequent egress of free biotin into the cytosol.

Thiamin represents another water-soluble vitamin whose particular path from plasma membrane to cytosol, for incorporation into apoenzymes, has not been elucidated (49). The lysosome may play a part in the intracellular route taken by thiamine and similar vitamins.

Other Systems

For every instance of receptor-mediated endocytosis involving lysosomes, there must be a means for the ligand involved to exit the lysosome. Some examples include the uptake of insulin by rat hepatocytes (107), the binding and internalization of human growth hormone by human lymphocytes (7), and the binding and internalization of epidermal growth factor by human epithe-

lioid carcinoma cells (50). Although these systems are not described in detail here, the movement of insulin, growth hormone, and epidermal growth factor out of the lysosome and into the cytosol must have approximately the same import for cellular nutrition as the availability of cholesterol or iron. In addition, for every unique product of macromolecular catabolism within the lysosome, such as thyroxine produced from thyroglobulin, there must be a system to effect movement across the lysosomal membrane. This transport step is often the missing link in a pathway of cellular nutrition otherwise well described.

SUMMARY AND CONCLUSIONS

A host of cellular nutrients are either synthesized within the cell itself or enter directly via transport across the plasma membrane. But a significant portion of the small molecules necessary for a cell's growth and metabolism require passage through the lysosome or a similar acidic vesicular compartment. These nutrients arrive in the lysosome by hydrolysis of macromolecules or by receptor-mediated endocytosis. They leave by carrier-mediated transport or, we speculate, by intermembrane transfer in the case of lipophilic substances. In either case, a vesicular membrane provides the key to communication between the lysosome and the cytosol, and that membrane now requires intensive study.

The success of future pursuits in this area will be aided by an increased recognition of the importance of the lysosome-to-cytosol step in cellular metabolism. For example, investigators might ask how iron gets out of endosomes, or how thyroxine gets out of lysosomes. In addition, naturally occurring mutations, or inborn errors of metabolism, provide invaluable opportunities to delineate a normal pathway, using the mutant for comparison. Physician and scientists alike can advance our knowledge of lysosomal membrane transport by increasing their collective index of suspicion that unknown lysosomal storage disorders might represent transport defects, rather than strictly enzymatic defects.

Literature Cited

1. Aisen, P., Listowsky, I. 1980. Iron transport and storage proteins. *Annu. Rev. Biochem.* 49:357–93
2. Anderson, R. G. W., Brown, M. S., Goldstein, J. L. 1977. Role of the coated endocytic vesicle in the uptake of receptor-bound low density lipoprotein in human fibroblasts. *Cell* 10:351–64
3. Anderson, R. G. W., Goldstein, J. L., Brown, M. S. 1976. Localization of low density lipoprotein receptors on plasma membrane of normal human fibroblasts and their absence in cells from a familial hypercholesterolemia homozygote. *Proc. Natl. Acad. Sci. USA* 73: 2434–38
4. Andersson, H. C., Tietze, F., Kohn, L. D., Kohn, A. D., Bernardini, I., et al. 1988. Carrier-mediated transport of monoiodotyrosine in thyroid cell lysosomes. *Pediatr. Res.* 23:387 (Abstr.)
5. Assmann, G., Fredrickson, D. S. 1983.

Acid lipase deficiency: Wolman's disease and cholesteryl ester storage. In *The Metabolic Basis of Inherited Disease,* ed. J. B. Stanbury, J. B., Wyngaarden, D. S., Fredrickson, J. L. Goldstein, M. S. Brown, pp. 803–19. New York: McGraw-Hill. 5th ed.

6. Baggiolini, M. 1972. The enzymes of the granules of polymorphonuclear leucocytes and their functions. *Enzyme* 13:132–60
7. Barazzone, P., Lesnick, M. A., Gorden, P., Van Obberghen, E., Carpenter, J.-L., et al. 1980. Binding, internalization, and lysosomal association of ^{125}I-human growth hormone in cultured human lymphocytes: A quantitative, morphological and biochemical study. *J. Cell. Biol.* 87:360–69
8. Barrett, A. J. 1969. Properties of lysosomal enzymes. In *Lysosomes in Biology and Pathology,* ed. J. T. Dingle, H. B. Fell, 2:245–312. Amsterdam: North Holland
9. Bell, F. P. 1975. Cholesterol exchange between microsomal, mitochondrial and erythrocyte membranes and its enhancement by cytosol. *Biochim. Biophys. Acta* 398:18–27
10. Bernar, J., Tietze, F., Kohn, L. D., Bernardini, I., Harper, G. S., et al. 1986. Characteristics of a lysosomal membrane transport system for tyrosine and other neurtral amino acids in rat thyroid cells. *J. Biol. Chem.* 261: 17107–12
11. Bird, S. J., Forster, S., Lloyd, J. B. 1987. Translocation of sugars into rat liver lysosomes. Evidence against a common carrier for D-glucose and D-ribose. *Biochem. J.* 245:929–31
12. Bothwell, T. H., Charlton, R. W., Motulsky, A. G. 1983. Idiopathic hemochromatosis. See Ref. 5, pp. 1269–98
13. Brady, R. O. 1983. Sphingomyelin lipidosis: Niemann-Pick disease. See Ref. 5, pp. 831–40
14. Bremner, I., Mehra, R. K. 1983. Metallothionein: Some aspects of its structure and function with special regard to its involvment in copper and zinc metabolism. *Chem. Scr.* 21:117–21
15. Brown, M. S., Goldstein, J. L. 1975. Regulation of the activity of the low density lipoprotein receptor in human fibroblasts. *Cell* 6:307–16
16. Brown, M. S., Goldstein, J. L. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232:34–47
17. Burton, P., Bloch, K. 1985. Studies on the mode of action of sterol carrier protein in the dehydration of 5-cholest-7-en-3β-ol. *J. Biol. Chem.* 260:7289–94
18. Camakaris, J., Danks, D. M., Ackland, L., Cartwright, E., Borger, P., et al. 1980. Altered copper metabolism in cultured cells from human Menke's syndrome and mottled mouse mutants. *Biochem. Genet.* 18:117–31
19. Casey, J. L., DiJeso, B., Rao, K., Klausner, R. D., Harford, J. B. 1988. Two genetic loci participate in the regulation by iron of the gene for the human transferrin receptor. *Proc. Natl. Acad. Sci. USA* 85:1787–91
20. Casey, J. L., Hentze, M. W., Koeller, D. M., Caughman, S. W., Rouault, T. A., et al. 1988. Iron-responsive elements: Regulatory RNA sequences that control mRNA levels and translation. *Science* 240:924–28
21. Casey, R. P., Hollemans, M., Tager, J. M. 1978. The permeability of the lysosomal membrane to small ions. *Biochim. Biophys. Acta* 508:15–26
22. Chan, W. Y., Cushing, W., Coffman, M. A., Rennert, O. M. 1980. Genetic expression of Wilson's disease in cell culture: A diagnostic marker. *Science* 208:299–300

22a. Christensen, H. N. 1988. Amino acid transport systems of lysosomes: Possible substitute utility of a surviving transport system for one congenitally defective or absent. *Biosci. Rep.* 8:121–29

23. Cooper, B. A., Rosenblatt, D. S. 1987. Inherited defects of vitamin B_{12} metabolism. *Annu. Rev. Nutr.* 7:291–320
24. Craft, D. V., Goss, N. H. 1982. Enzymatic degradation of protein-bound biotin by biotinidase. *Fed. Proc.* 41: 742–53
25. Danks, D. M. 1983. Hereditary disorders of copper metabolism in Wilson's disease and Menke's disease. See Ref. 5, pp. 1251–68
26. Dawidowicz, E. A. 1987. Dynamics of membrane lipid metabolism and turnover. *Annu. Rev. Biochem.* 56:43–61
27. de Duve, C., Wattiaux, R. 1966. Functions of lysosomes. *Annu. Rev. Physiol.* 28:435–92
28. Docherty, K., Brenchley, G. V., Hales, C. N. 1979. The permeability of rat liver lysosomes to sugars. Evidence for carrier-mediated facilitated diffusion. *Biochem. J.* 178:362–66
29. Dunn, M. A., Blalock, T. L., Cousins, R. J. 1987. Metallothionein (42525A). *Proc. Soc. Exp. Biol. Med.* 185:107–19
30. Ehrenreich, B. A., Cohn, Z. A. 1969. The fate of peptides pinocytosed by

macrophages *in vitro*. *J. Exp. Med.* 129:227–45
31. Ettlinger, J. D., Goldberg, A. L. 1977. A soluble ATP-dependent proteolytic system for the degradation of abnormal proteins in reticulocytes. *Proc. Natl. Acad. Sci. USA* 74:54–58
32. Fiedler-Nagy, C., Rowley, G. R., Coffey, J. W., Miller, O. W. 1979. Binding of vitamin B_{12}-rat transcobalamin II and free vitamin B_{12} to plasma membranes isolated from rat liver. *Br. J. Haematol.* 31:311–21
33. Friedman, P. A., Shia, M. A., Wallace, J. K. 1977. A saturable high affinity binding site for transcobalamin II–vitamin B_{12} complexes in human placental membrane preparations. *J. Clin. Invest.* 59:51–58
34. Gahl, W. A. 1986. Cystinosis coming of age. *Adv. Pediatr.* 33:95–126
35. Gahl, W. A., Bashan, N., Tietze, F., Bernardini, I., Schulman, J. D. 1982. Cystine transport is defective in isolated leukocyte lysosomes from patients with cystinosis. *Science* 217:1263–65
36. Gahl, W. A., Bashan, N., Tietze, F., Schulman, J. D. 1984. Lysosomal cystine counter-transport in heterozygotes for cystinosis. *Am. J. Hum. Genet.* 36:277–82
37. Gahl, W. A., Reed, G. F., Thoene, J. G., Schulman, J. D., Rizzo, W. B., et al. 1987. Cysteamine therapy for children with nephropathic cystinosis. *New Engl. J. Med.* 316:971–77
38. Gahl, W. A., Renlund, M., Thoene, J. G. 1989. Lysosomal transport disorders---cystinosis and sialic acid storage disorders. In *The Metabolic Basis of Inherited Disease,* ed. C. R. Scriver, A. Beaudet, W. Sly, D. Valle. New York: McGraw-Hill. 6th ed. In press
39. Gahl, W. A., Tietze, F., Bashan, N., Bernardini, I., Raiford, D., et al. 1983. Characteristics of cystine counter-transport in normal and cystinotic lysosome-rich leucocyte granular fractions. *Biochem. J.* 216:393–400
40. Gahl, W. A., Tietze, F., Bashan, N., Steinherz, R., Schulman, J. D. 1982. Defective cystine exodus from isolated lysosome-rich fractions of cystinotic leucocytes. *J. Biol. Chem.* 257:9570–75
41. Gahl, W. A., Tietze, F., Butler, J. D., Schulman, J. D. 1985. Cysteamine depletes cystinotic leucocyte granular fractions of cystine by the mechanism of disulphide interchange. *Biochem. J.* 228:545–50
42. Gil, G., Faust, J. R., Chin, D. J., Goldstein, J. L., Brown, M. S. 1985. Membrane-bound domain of HMG CoA reductase is required for sterol-enhanced degradation of the enzyme. *Cell* 41:249–58
43. Goldfischer, S., Popper, H., Sternlieb, I. 1980. The significance of variations in the distribution of copper in liver disease. *Am. J. Pathol.* 99:715–24
44. Goldman, R., Kaplan, A. 1973. Rupture of rat liver lysosomes mediated by L-amino acid esters. *Biochim. Biophys. Acta* 318:205–16
45. Goldstein, J. L., Basu, S. K., Brunschede, G. Y., Brown, M. S. 1976. Release of low density lipoprotein from its cell surface receptor by sulfated glycosaminoglycans. *Cell* 7:85–95
46. Goldstein, J. S., Brown, M. S. 1983. Familial hypercholesterolemia. See Ref. 5, pp. 672–712
47. Gordon, A. H. 1969. The role of lysosomes in protein catabolism. In *Lysosomes in Biology and Pathology,* ed. J. T. Dingle, H. B. Fell, 2:89–137. Amsterdam: North Holland
48. Greene, A. A., Clark, K. F., Smith, M. L., Schneider, J. A. 1987. Cystine exodus from normal leucocytes is stimulated by Mg ATP. *Biochem. J.* 246:547–49
49. Haas, R. H. 1988. Thiamin and the brain. *Annu. Rev. Nutr.* 8:483–515
50. Haigler, H., Ash, J. F., Singer, S. J., Cohen, S. 1978. Visualization by fluorescence of the binding and internalization of epidermal growth factor in human carcinoma cells A-431. *Proc. Natl. Acad. Sci USA* 75:3317–21
51. Hall, C. A., Green-Colligan, P. D., Begley, J. A. 1985. The metabolism of cobalamin bound to transcobalamin II and to glycoproteins that bind Cbl in HepG2 cells (human hepatoma). *J. Cell. Physiol.* 124:507–15
52. Hanover, J. A., Dickson, R. B. 1985. Transferrin: Receptor-mediated endocytosis and iron delivery. In *Receptor-Mediated Endocytosis,* ed. I. Pastan, M. C. Willingham, pp. 131–61. New York: Plenum
53. Harper, G. S., Kohn, L. D., Bernardini, I., Bernar, J., Tietze, F., et al. 1988. Thyrotropin stimulates lysosomal tyrosine transport in rat FRTL-5 thyroid cells. *J. Biol. Chem.* 263:9320–25
54. Hasilik, A., Waheed, A., von Figura, K. 1981. Enzymatic phosphorylation of lysosomal enzymes in the presence of UDP-*N*-acetylglucosamine. Absence of the activity in I-cell fibroblasts. *Biochem. Biophys. Res. Commun.* 98:761–67
55. Held, D. D., Hoekstra, W. G. 1979. *In vitro* degradation of metallothionein by

rat-liver lysosomes. *Fed. Proc.* 38:734–74

56. Hentze, M. W., Caughman, S. W., Rouault, T. A., Barriocanal, J. G., Dancis, A., et al. 1987. Identification of the iron-responsive element for the translational regulation of human ferritin mRNA. *Science* 238:1570–73
57. Hentze, M. W., Rouault, T. A., Caughman, S. W., Dancis, A., Harford, J. B., et al. 1987. A *cis*-acting element is necessary and sufficient for translational regulation of human ferritin expression in response to iron. *Proc. Natl. Acad. Sci. USA* 84:6730–34
58. Hershko, A., Ciechanover, A. 1982. Mechanisms of intracellular protein breakdown. *Annu. Rev. Biochem.* 51: 335–64
59. Hommes, F. A. 1986. Biotin. *World Rev. Nutr. Diet.* 48:34–84
60. Horn, N., Jensen, O. A. 1980. Menkes' syndrome: Subcellular distribution of copper determined by an ultrastructural histochemical technique. *Ultrastruct. Pathol.* 1:237–42
61. Huebers, H. A., Finch, C. A. 1987. The physiology of transferrin and transferrin receptors. *Physiol. Rev.* 67:520–82
62. Jonas, A. J., Smith, M. L., Allison, W. S., Laikind, P. K., Greene, A. A., et al. 1983. Proton-translocating ATPase and lysosomal cystine transport. *J. Biol. Chem.* 258:11727–30
63. Jonas, A. J., Smith, M. L., Schneider, J. A. 1982. ATP-dependent lysosomal cystine efflux is defective in cystinosis. *J. Biol. Chem.* 257:13185–88
64. Kaiser-Kupfer, M. I., Fujikawa, L., Kuwabara, T., Jain, S., Gahl, W. A. 1987. Removal of corneal crystals by topical cysteamine in nephropathic cystinosis. *New Engl. J. Med.* 316:775–79
65. Kirschke, H., Wiederanders, B. 1987. Lysosomal proteinases. *Acta Histochem.* 82:2–4
66. Kruth, H. S., Comly, M. E., Butler, J. D., Vanier, M. T., Fink, J. K., et al. 1986. Type-C Niemann-Pick disease. Abnormal metabolism of low density lipoprotein in homozygous and heterozygous fibroblasts. *J. Biol. Chem.* 261:16769–74
67. La Russo, N. F., Fowler, S. 1979. Coordinate secretion of acid hydrolases in rat bile. Hepatocyte exocytosis of lysosomal protein? *J. Clin. Invest.* 64:948–54
68. Liscum, L., Faust, J. R. 1987. Low-density lipoprotein (LDL)–mediated suppression of cholesterol synthesis and LDL uptake is defective in Niemann-Pick Type-C fibroblasts. *J. Biol. Chem.* 262:17002–8
69. Lloyd, J. B. 1969. Studies on the permeability of rat liver lysosomes to carbohydrates. *Biochem. J.* 115:703–7
70. Lucy, J. A. 1969. Lysosomal membranes. In *Lysosomes in Biology and Pathology*, ed. J. T. Dingle, H. B. Fell, 2:313–41. Amsterdam: North Holland
71. Luskey, K. L., Faust, J. R., Chin, D. J., Brown, M. S. Goldstein, J. L. 1983. Amplification of the gene for 3-hydroxy-3-methylglutaryl coenzyme A reductase, but not for the 53-kDa protein, in UT-1 cells. *J. Biol. Chem.* 258:8462–69
72. McKusick, V. A., Neufeld, E. F. 1983. The mucopolysaccharide storage diseases. See Ref. 5, pp. 751–77
73. Maguire, G. A., Docherty, K., Hales, C. N. 1983. Sugar transport in rat liver lysosomes. Direct demonstration using labelled sugars. *Biochem. J.* 212:211–18
74. Marsh, M., Bolzau, E., Helenius, A. 1983. Penetration of Semliki Forest Virus from acidic prelysosomal vacuoles. *Cell* 32:931–40
75. Mason, R. W. 1986. Species variations amongst lysosomal cysteine proteinases. *Biomed. Biochim. Acta* 45:1433–40
76. Maxfield, F. R. 1982. Weak bases and ionophores rapidly and reversibly raise the pH of endocytic vesicles in cultured mouse fibroblasts. *J. Cell Biol.* 95:676–81
77. Maziere, C., Maziere, J. C., Mora, L., Lageron, A., Polonovski, C., et al. 1987. Alterations in cholesterol metabolism in cultured fibroblasts from patients with Niemann-Pick disease Type-C. *J. Inherit. Metab. Dis.* 10:339–46
78. Munro, H. N., Linder, M. C. 1978. Ferritin: Structure, biosynthesis, and role in iron metabolism. *Physiol. Rev.* 58:317–96
79. Neufeld, E. F. 1983. The William Allan Memorial Award Address: Cell mixing and its sequelae. *Am. J. Hum. Genet.* 35:1081–85
80. Nishimura, Y., Kato, K. 1987. Intracellular transport and processing of lysosomal 21 cathepsin B. *Biochem. Biophys. Res. Commun.* 148:254–59
81. Nishimura, Y., Kato, K. 1987. Intracellular transport and processing of lysosomal cathepsin H. *Biochem. Biophys. Res. Commun.* 148:329–34
82. Noland, J., Arebalo, R. E., Hansbury, E., Scallen, T. J. 1980. Purification and properties of sterol carrier protein. *J. Biol. Chem.* 255:4282–89
83. Pearse, B. 1976. Clathrin: A unique protein associated with intracellular transfer

of membrane by coated vesicles. *Proc. Natl. Acad. Sci. USA* 73:1255–59
84. Pentchev, P. G., Comly, M. E., Kruth, H. S., Tokoro, T., Butler, J., et al. 1987. Group C Niemann-Pick disease: Faulty regulation of low-density lipoprotein uptake and cholesterol storage in cultured fibroblasts. *FASEB J.* 1:40–45
85. Pentchev, P. G., Comly, M. E., Kruth, H. S., Vanier, M. T., Wenger, D. A., et al. 1985. A defect in cholesterol esterification in Niemann-Pick disease (type-C) patients. *Proc. Natl. Acad. Sci. USA* 82:8247–51
86. Pentchev, P. G., Kruth, H. S., Comly, M. E., Butler, J. D., Vanier, M. T., et al. 1986. Type-C Niemann-Pick disease. A parallel loss of regulatory responses in both the uptake and esterification of low-density lipoprotein–derived cholesterol in cultured fibroblasts. *J. Biol. Chem.* 261:16775–80
87. Pisoni, R., Flickinger, K., Thoene, J., Christensen, H. 1987. Characterization of carrier-mediated transport systems for small neutral amino acids in human fibroblast lysosomes. *J. Biol. Chem.* 262:6010–17
88. Pisoni, R. L., Thoene, J. G. 1988. Demonstration of a nucleoside transport system in lysosomes of human fibroblasts. *FASEB J.* 2:1743 (Abstr.)
89. Pisoni, R. L., Thoene, J. G., Christensen, H. N. 1985. Detection and characterization of carrier-mediated cationic amino acid transport in lysosomes of normal and cystinotic human fibroblasts. *J. Biol. Chem.* 260:4791–98
90. Pisoni, R., Thoene, J., Lemons, R., Christensen, H. 1987. Important differences in cationic amino acid transport by lysosomal system c and system y^+ of the human fibroblast. *J. Biol. Chem.* 262:15011–18
91. Reeves, J. P. 1979. Accumulation of amino acids by lysosomes incubated with amino acid methyl esters. *J. Biol. Chem* 254:8914–21
92. Reinhart, M. P., Billheimer, J. T., Faust, J. R., Gaylor, J. L. 1987. Subcellular localization of the enzymes of cholesterol biosynthesis and metabolism in rat liver. *J. Biol. Chem.* 262:9649–55
93. Reitman, M. L., Kornfeld, S. 1981. UDP-*N*-Acetylglucosamine: Glycoprotein *N*-acetylglucosamine-1-phosphotransferase: Proposed enzyme for the phosphorylation of the high mannose oligosaccharide units of lysosomal enzymes. *J. Biol. Chem.* 256:4275–81
94. Renlund, M., Tietze, F., Gahl, W. A. 1986. Defective sialic acid egress from isolated fibroblast lysosomes of patients with Salla disease. *Science* 232:759–62
95. Rome, L. H., Hill, D. F. 1986. Lysosomal degradation of glycoproteins and glycosaminoglycans. Efflux and recycling of sulphate and *N*-acetylhexosamines. *Biochem. J.* 235:707–13
96. Rosenblatt, D. S., Hosack, A., Matiaszuk, N. V., Cooper, B. A., Laframboise, R. 1985. Defect in vitamin B_{12} release from lysosomes: Newly described inborn error of vitamin B_{12} metabolism. *Science* 228:1319–21
97. Rosenblatt, D. S., Laframboise, R., Pichette, J., Langerin, P., Cooper, B. A., et al. 1986. New disorder of vitamin B_{12} metabolism (cobalamin F) presenting as methylmalonic aciduria. *Pediatrics* 78:51–54
98. Said, H. M., Redha, R. 1987. A carrier-mediated system for transport of biotin in rat intestine *in vitro*. *Am. J. Physiol.* 15:52–5
99. Scallen, J., Pastuszyn, A., Noland, J., Chanderbhan, R., Kharroubi, A., et al. 1985. Sterol carrier and lipid transfer proteins. *Chem. Phys. Lipids* 38:239–61
100. Schneider, J. A., Schulman, J. D., Seegmiller, J. E. 1978. Cystinosis and the Fanconi syndrome. In *The Metabolic Basis of Inherited Disease*, ed. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, pp. 1660–82. New York: McGraw-Hill. 4th ed.
101. Smith, M. L., Greene, A. A., Potashnik, R., Mendoza, S. A., Schneider, J. A. 1987. Lysosomal cystine transport. Effect of intralysosomal pH and membrane potential. *J. Biol. Chem.* 262: 1244–53
102. Srikantaiah, V., Hansbury, E., Loughran, D., Scallen, J. 1976. Purification and properties of sterol carrier protein 1. *J. Biol. Chem.* 251:5496–5504
103. Steinherz, R., Tietze, F., Gahl, W. A., Triche, T. J., Chiang, H., et al. 1982. Cystine accumulation and clearance by normal and cystinotic leukocytes exposed to cystine dimethylester. *Proc. Natl. Acad. Sci. USA* 79:4446–50
104. Sternlieb, I. 1980. Copper and the liver. *Gastroenterology* 78:1615–28
105. Sternlieb, I., Van den Hamer, C. J. A., Morell, A. G., Alpert, S., Gregoriadis, G., et al. 1973. Lysosomal defect of hepatic copper excretion in Wilson's disease (hepatolenticular degeneration). *Gastroenterology* 64:99–105
106. Tanner, M. S. 1986. Indian childhood cirrhosis. In *Recent Advances in*

Paediatrics, ed. R. Meadow, 8:103–20. New York: Churchill Livingstone
107. Terris, S., Steiner, D. F. 1975. Binding and degradation of ^{125}I-insulin by rat hepatocytes. *J. Biol. Chem.* 250:8389–98
108. Thoene, J. G., Lemons, R. 1980. Modulation of the intracellular cystine content of cystinotic fibroblasts by extracellular albumin. *Pediatr. Res.* 14:785–87
109. Thoene, J. G., Lemons, R. 1982. Cystine accumulation in cystinotic fibroblasts from free and protein-linked cystine but not cysteine. *Biochem. J.* 208:823–30
110. Thoene, J. G., Lemons, R., Boskovich, S., Borysko, K. 1985. Inhibitors of protein synthesis also inhibit lysosomal proteolysis: Studies using cystinotic fibroblasts. *J. Clin. Invest.* 75:370–76
111. Thoene, J. G., Oshima, R. G., Crawhall, J. C., Olson, D. L., Schneider, J. A. 1976. Cystinosis: Intracellular cystine depletion by aminothiols in vitro and in vivo. *J. Clin. Invest.* 58:180–89
112. Thoene, J. G., Oshima, R. G., Ritchie, D. G., Schneider, J. A. 1977. Cystinotic fibroblasts accumulate cystine from intracellular protein degradation. *Proc. Natl. Acad. Sci. USA* 74:4505–7
113. Thomas, G. H., Scocca, J., Miller, C. S., Reynolds, L. W. 1985. Accumulation of *N*-acetylneuraminic acid (sialic acid) in human fibroblasts cultured in the presence of *N*-acetylmannosamine. *Biochim. Biophys. Acta* 846:37–43
114. Tietze, F., Renlund, M., Harper, G., Thomas, G., Gahl, W. A. 1986. Characteristics of lysosomal free sialic acid transport defective in infantile free sialic acid storage disease. *Am. J. Hum. Genet.* 39:A21
115. Trowbridge, I. S., Newman, R. A., Domingo, D. L., Sauvage, C. 1984. Transferrin receptors: Structure and function. *Biochem. Pharmacol.* 33:925–32
116. Tsuji, T., Karasek, M. 1983. A procedure for the isolation of primary cultures of melanocytes from newborn and adult human skin. *J. Invest. Dermatol.* 81:179–80
116a. Vassiliadis, A., Rosenblatt, D. S., Cooper, B. A., Bergeron, J. J. M. 1988. EM autoradiography of cbl F fibroblasts: Demonstration of vitamin B_{12} in lysosomes. *Am. J. Hum. Genet.* 43:A17
117. Vesely, D. L., Kemp, S. F., Elders, M. J. 1987. Isolation of a biotin receptor from hepatic plasma membranes. *Biochem. Biophys. Res. Commun.* 143:913–16
118. Winge, D. R. 1984. Normal physiology of copper metabolism. *Semin. Liv. Dis.* 4:239–51
119. Witkop, C. J., Quevedo, W. C. Jr., Fitzpatrick, T. B. 1983. Albinism and other disorders of pigment metabolism. See Ref. 5, pp. 301–46
120. Wolf, B., Grier, R. E., McVoy, J. R. S., Heard, G. S. 1985. Biotinidase deficiency: A novel vitamin recycling defect. *J. Inherit. Metab. Dis.* 8(Suppl. 1):53–58
121. Yaegle, P. L., Young, J. E. 1986. Factors contributing to the distribution of cholesterol among phospholipid vesicles. *J. Biol. Chem.* 261:8175–81
122. Youngdahl-Turner, P., Rosenberg, L. E., Allen, R. H. 1978. Binding and uptake of transcobalamin II by human fibroblasts. *J. Clin. Invest.* 61:133–41