Morphologic and Biochemical Abnormalities of Kidney Lysosomes in Mice With an Inherited Albinism

M. Meisler, PhD, J. Levy, MS, F. Sansone, PhD, and M. Gordon, PhD

The light-ear mutation in the mouse may serve as a useful model for the human inherited oculocutaneous albinisms such as the Hermansky-Pudlak and Chédiak-Higashi syndromes. The authors have investigated the kidney lysosomes of le/le mutant mice by histochemical methods. A striking increase in the staining reaction for the lysosomal enzymes β-galactosidase and acid-phosphatase was evident in kidney cortex of the mutant mice, in comparison with +/+ controls. The lysosomal protease, cathepsin C, is also found to be elevated in the mutant. By light microscopy, there appeared to be an increase in the number of lysosomes in mutant kidney. Electron microscopy revealed the presence of large, multilamellar granules in proximal tubule cells. Analysis of sedimentation through sucrose gradients demonstrated the presence of a low-density population of lysosomes in the mutant kidney. In addition, a striking accumulation of ceroidlike pigment was observed. The molecular lesions responsible for the melanolysosomal syndromes in mice and man are still unidentified. (Am J Pathol 1980, 101:581-594)

IN THE OCULOCUTANEOUS ALBINISM SYNDROMES of man, hypopigmentation is caused by abnormalities in melanosome structure, rather than by the loss of pigment biosynthetic enzymes. In the Hermansky–Pudlak and the Chédiak–Higashi syndromes, for example, abnormalities of melanosomes, lysosomes, and other organelles have been described.[1] The nature of the primary defect in these disorders is not known.

An analogous series of inherited defects has been described in the mouse. Mutant alleles at 9 distinct loci are known to produce dilution of pigmentation and lysosomal dysfunction.[2] The best studied of the mouse mutants is the beige mouse, which has been considered a model for the Chédiak–Higashi syndrome. Beige mice are characterized by giant granules in many cell types, defective excretion of lysosomes from the kidney, platelet abnormalities, and accumulation of ceroid pigment in several tissues.[3]

The light-ear (*le*) mutation at a locus on mouse chromosome 9 appears to be another example of this class of disorders. We have described the defective urinary secretion of β-galactosidase in this mutant, which results in the accumulation of the enzyme in kidneys of homozygous male animals.[4]

From the Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, the Department of Anatomy, SUNY School of Medicine, Buffalo, New York, and the CUNY Biomedical Program, New York, New York.

Supported by USPHS Grant GM-24872 and by the National Foundation/March of Dimes.

Accepted for publication June 24, 1980.

Address reprint requests to Miriam H. Meisler, PhD, Department of Human Genetics, University of Michigan Medical School, 1137 E. Catherine Street, Ann Arbor, MI 48109.

0002-9440/80/1209-0581$01.00

In this report, we describe histologic studies of the mutant kidney. Our results demonstrate additional similarities between the light-ear mice and the human disorders described above.

Microscopic studies of retinal melanosomes from *le/le* mice have previously been reported.[5] These authors observed reduced numbers of melanosomes in choroidal melanocytes and in retinal pigment epithelial cells. The melanosomes of melanocytes were on the average larger, more heterogeneous in size, and less densely packed than in *le/+* controls. We have studied lysosome structure in kidneys of male *le/le* mice because the accumulation of the lysosomal enzyme β-galactosidase is most pronounced in this tissue, reaching levels 4 to 5 times higher than in controls.

Materials and Methods

Animals

Breeding pairs of C57BL/6J-*le/le* mice were originally obtained from Dr. Verne Chapman, Roswell Park Memorial Laboratory, Buffalo, New York, and have been bred in our laboratory since 1975. C57BL/6J controls were purchased from the Jackson Laboratory, Bar Harbor, Maine. Male animals were used for these studies, since testosterone induces lysosome biosynthesis in mouse kidney and amplifies the effects of the le mutation.[4]

Light Microscopy

Kidneys from 1-year-old male *le/le* and +/+ mice, rolled in talcum powder, and quick-frozen in Freon, were cooled to −170 C with liquid nitrogen. Ten-micron cryostat sections were fixed in cold buffered neutral formalin for 5–10 minutes, rinsed, and stained at 37 C for 10, 20, or 30 minutes. Control sections were incubated in complete medium minus substrate. β-Galactosidase activity was visualized with the use of the substrate 5-bromo-4-chloro-3-indoyl-β-D-galactoside.[6] The reaction mixture for detection of acid phosphatase activity contained 10 mM β-glycerophosphate and 4 mM lead nitrate in 50 mM acetate buffer, pH 5.0.[7] After incubation, sections were immersed in ammonium disulfide for 1–2 minutes to develop the lead sulfide reaction.

Electron Microscopy

Blocks of kidney cortex smaller than 1 cu mm were placed in 1.5% glutaraldehyde, 0.1 M cacodylate buffer, pH 7.2, at 4 C. After fixation for 15 minutes, the tissue blocks were washed overnight in the same buffer containing 0.4 M sucrose. Specimens were stained for acid phosphatase activity as described above. After incubation for either 15 or 30 minutes tissue blocks were refixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Spurr resin (Polysciences). One-micron plastic sections were used to locate proximal convoluted tubules. Thin sections were cut on an LKB ultramicrotome and examined in a JEOL 100B electron microscope either without additional staining or after staining with uranyl and lead salts.

Subcellular Fractionation

Kidneys were minced and homogenized in 0.25 M sucrose, 0.05 M Tris, pH 7.5, with 10 strokes of a loose-fitting Teflon pestle (clearance 0.015 inches) rotating at 1000 rpm. Centrifugation of the particulate fraction through discontinuous sucrose gradients (17 ml total volume) was carried out as described by Lusis et al [8] in 0.05 M Tris buffer, pH 7.5. Gradi-

ents were centrifuged for 30 minutes at 15,000 rpm in the SW 27.1 rotor; 0.7 ml fractions were collected. β-Galactosidase activity of gradient fractions was measured in 0.1 M citrate buffer, pH 3.5, with the substrate *p*-nitrophenyl-β-D-galactoside (Sigma) as previously described.[9]

Cathepsin C was assayed by a modification of the method of McDonald et al.[10] Kidney homogenates (5–10%) were centrifuged for 20 minutes at 5,000*g*. An aliquot of the supernate (25–50 μl) was incubated with 175 μl of substrate solution containing 0.1 mM ser-tyr-β-napthylamide (Sigma), 1 mM dithiothreitol, 5 mM NaCl, and 70 mM succinate, pH 4.0. The reaction was stopped by the addition of 1.8 ml of 0.05 M Tris HCl, pH 8.8. Fluorescence was measured in a Turner Fluorimeter with primary filter 7-60 and secondary filter 2A. Protein was determined by the method of Lowry [11] with bovine serum albumin as standard.

Results

Light Microscopy

The specific activity of β-galactosidase in homogenates of kidney from male *le/le* mice is 4 times higher than in +/+ controls.[4] When kidney slices from mutant and +/+ animals were stained for β-galactosidase activity, the localization of accumulated β-galactosidase to the kidney cortex was clearly evident (Figure 1). In *le/le* kidney, staining was visible after 10 minutes of incubation and increased in intensity for 30 minutes. In contrast, a 30-minute reaction was necessary to visualize β-galactosidase in +/+ kidney (Figure 2); the staining in +/+ kidney cortex was much less intense than in the mutant. No reaction was visible in the medulla of either genotype. Control sections incubated without substrate were negative.

To provide a second lysosome marker, acid phosphatase activity was also stained in kidney sections. With this stain, an increase in the number and size of acid-phosphatase–positive granules was apparent in *le/le* mice (Figures 3 and 4). After 30 minutes of staining, the lysosomes appeared as discrete, punctuate, dark granules in the apices of the proximal convoluted tubules (Figure 3). In the +/+ kidney, fewer lysosomal granules were discernible (Figure 4). Prolongation of incubation beyond 30 minutes did not enhance the phosphatase reaction in either genotype. Control sections incubated without β-glycerophosphate were completely negative.

Electron Microscopy

Since a high concentration of lysosomes was evident in the region of proximal tubule cells, these cells were examined in greater detail. Figures 5 and 6 represent portions of the proximal convoluted epithelium, which included both the brush border and basal lamina in the plane of section. Because lysosomes congregate at the cell apex, views utilizing the bases of

the brush borders as markers were selected for comparison. In control mice, lysosomes varied in appearance from small dense bodies (Figure 5) to organelles containing whorls of myelin figures (Figure 7). Examination of the mutant kidney commonly demonstrate greater numbers of lysosomes (Figure 6). Some are similar to those seen in the +/+ mice, but many are much larger. Some lysosomes in *le/le* kidneys contain elaborate fine-structural features. A common variant was a lysosome with a complex, internal honeycomb construct (Figure 8). Occasionally crystals of reaction product were located within the organelles, but usually the reacted lysosomes had a uniform density, where the solid regions, membranes, and other structural components were enhanced by the lead reaction product. We conclude that the lysosomes of the mutant mice were structurally more complex than those of +/+ kidney. Mutant kidney consistently contained numerous endocytotic channels and vesicles just beneath the brush border (Figure 6) in contrast with +/+ kidneys (Figure 5). It was not feasible to produce a quantitative comparison of lysosome numbers in the two genotypes, since they were numerous in some planes of section, while in others there were relatively few organelles.

Exocytosis of Lysosomes

In some electron-microscopic sections of the apical portion of the proximal tubule cell, acid-phosphatase–positive granules are visible in close association with the microvilli (not shown). Membrane-bound vesicles are also apparent within the lumen. These observations are consistent with hypothesis that intact lysosomes are extruded through the microvilli, as suggested by Koenig et al.[12] A lesion affecting this exocytotic process would account for the reduced urinary secretion of lysosomal enzymes in *le/le* mice.

Accumulation of Ceroidlike Pigment in Kidney Cortex

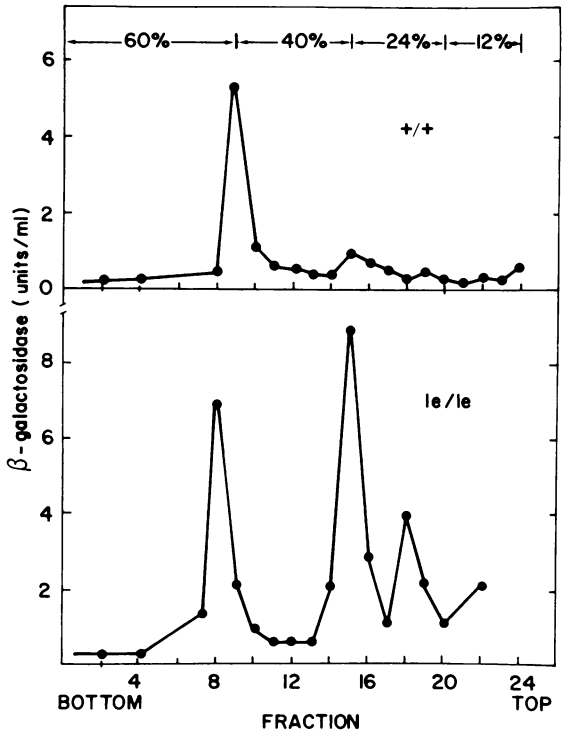
Frozen sections of kidney were examined by fluorescence microscopy.[15] An intense yellow-green autofluorescence was present in proximal tubules of the le/le mutant (Figure 9). In contrast, in the sections from +/+ controls, the only fluorescent material was present as small granules within tubules having the appearance of distal tubules; the proximal tubule cells were free of fluorescence. The striking difference between mutant and control sections suggests that large quantities of material are stored. The appearance of this material resembles that of ceroidlike pigment, which accumulates in human tissues in related disorders.[1]

Low Density of Lysosomes in Mutant Kidneys

A crude lysosomal fraction was prepared from kidney homogenates and analyzed by sedimentation through a discontinuous sucrose gradient (Text-figure 1). In +/+ kidney, the major portion of the β-galactosidase activity sedimented to the interfaces 60/40 and 40/24, in agreement with an earlier report.[8] However, in le/le preparations there was a consistent shift of β-galactosidase activity toward the fractions of lower density, predominantly to the 40/24 interface but also to the 24/12 interface. The distribution of a second lysosomal enzyme, β-glucuronidase, was parallel to the β-galactosidase distribution (not shown). These shifts in position of lysosomal enzymes indicate that the mutant kidneys contain a population of lysosomes with abnormally low density. This shift in density may reflect the accumulated glycolipids [13] and ceroidlike pigment in this tissue.

Accumulation of a Lysosomal Protease in Mutant Kidney

Until now, enzymatic studies of the lysosomal abnormalities in mutant mice have been limited to one class of hydrolase, the glycosidases. To test



TEXT-FIGURE 1—Sucrose gradient sedimentation of lysosomes for mutant and control kidney. A particulate fraction from kidney homogenates was sedimented through a 16.5-ml discontinuous sucrose gradient as described in Materials and Methods. Fractions (0.7 ml) were collected and assayed for β-galactosidase activity.

the generality of the storage defect, we measured the activity of the lysosomal protease cathepsin C in homogenates of +/+ and *le/le* kidneys. There is a significant increase in cathepsin C activity in *le/le* kidneys. In age-matched +/+ males, the activity of kidney homogenates was 0.2 ± 0.02 nmoles/min/mg protein (n=7), while in le/le kidney the enzyme activity was 1.4 ± 0.2 nmoles/min/mg protein (n=6). This observation supports the concept of a generalized lysosome defect in this mutant.

Discussion

We have described 4 new features of the light-ear mutation in the mouse: an increase in the number of moderately enlarged lysosomes in proximal tubules cells of the kidney, the reduced average density of lysosomes in this tissue, accumulation of a lysosomal protease in mutant kidneys, and the accumulation of fluorescent ceroidlike pigment in tubule cells. The accumulated β-galactosidase in *le/le* kidney was localized to the kidney cortex by histochemical staining, and the special involvement of proximal tubule cells is indicated by the localization of the autofluorescent material. However, we did not observe increased acid phosphatase activity when *le/le* kidney homogenates were assayed with β-glycerol phosphate as substrate. The enhanced staining in this case may be related to altered permeability of the lysosome.

The le mutation and the 7 related pigment mutants may be described as "organellar" mutants with pleiotropic effects on lysosomes, melanosomes, and other intracellular granules. The morphologic studies place the *le/le* mutation in the group [16] which is characterized by lysosomes ranging in size up to three times the normal dimensions, but distinctly smaller than the "giant" organelles found in tissues of the beige mouse. The density of lysosomes in other mutants has not yet been studied; it will be of interest to compare glycolipid metabolism and lysosome density in other genetic variants.

The striking accumulation of ceroidlike pigment in kidney tubules supports our view that a defect in exocytosis is the primary lesion in the le/le mutant. A similar finding was reported in tissues of the beige mouse.[14] The known similarities between the light-ear mouse, the beige mouse, and the human oculocutaneous albinisms include hypopigmentation, intralysosomal storage of fluorescent compounds, and abnormal metabolism of other intracellular granules. Further characterization of these mouse models may contribute to the identification of the primary effect in these organellar disorders.

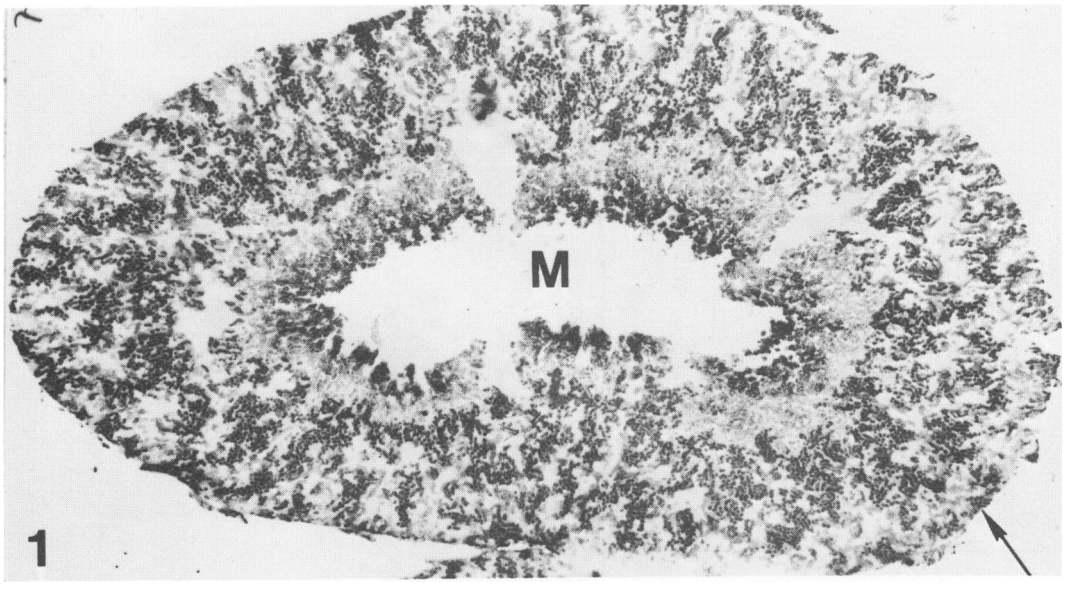
References

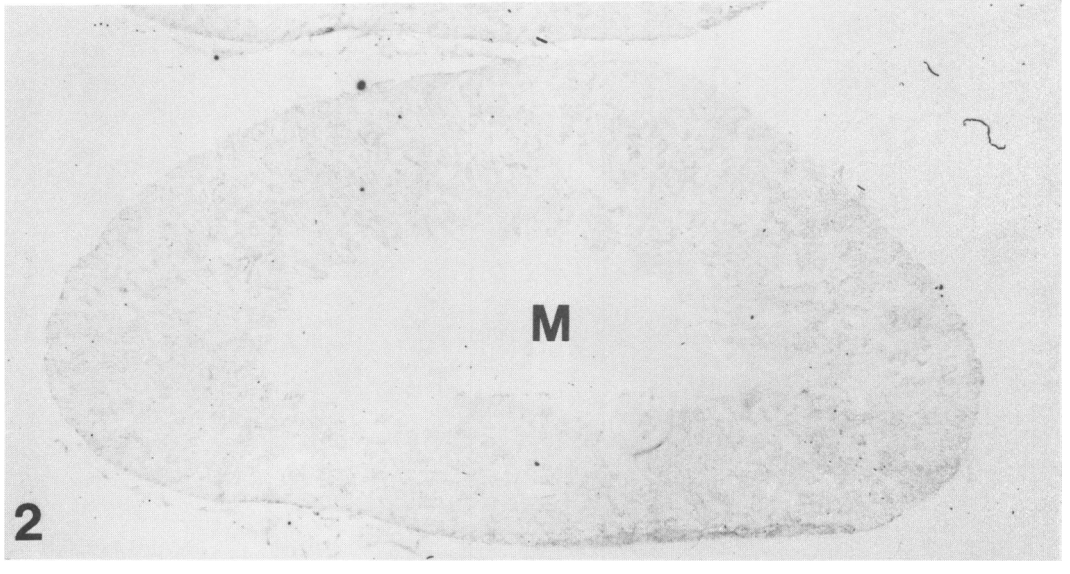
1. Witkop CJ, Quevado WC, Fizpatrick TB: Albinism, The Metabolic Basis of Inherited Disease. Edited by JB Stanbury, JB Wyngaarden, DS Fredrickson. 4th edition. New York, McGraw-Hill, 1978, pp 286–306
2. Novak EK, Swank RT: Lysosomal dysfunctions associated with mutations at mouse pigment genes. Genetics 1979, 92:189–204
3. Brandt EJ, Swank RT, Novak EK: The murine Chediak–Higashi mutation and other murine pigmentation mutations: A review, Immunological Defects in Laboratory Animals. Edited by ME Gershwin. New York, Plenum Press, 1980
4. Meisler MH: Synthesis and secretion of kidney β-galactosidase in mutant le/le mice. J Biol Chem 1978, 253:3129–3134
5. LaVail MM, Sidman RL: C57BL/6J Mice with inherited retinal degeneration. Arch Opthalmol 1974, 91:394–400
6. Felton J, Meisler M, Paigen K: A locus determining β-galactosidase activity in the mouse. J Biol Chem 1974, 249:3267–3272
7. Gomorri G: Microscopic Histochemistry. Chicago, University of Chicago Press, 1952
8. Lusis AJ, Breen GAM, Paigen K: Nongenetic heterogeneity of mouse β-galactosidase. J Biol Chem 1977, 252:4613–5618
9. Meisler MH, Paigen K: Coordinated development of β-glucuronidase and β-galactosidase in mouse organs. Science 1972, 177:894–896
10. McDonald JK, Ellis S, Reilly T: Properties of dipeptidyl arylamidase I of the pituitary. J Biol Chem 1966, 241:1494–1501
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem 1951, 193:265–275
12. Koenig H, Goldstone A, Hughes C: Lysosomal enzymuria in the testosterone-treated mouse. Lab Invest 1978, 39:329–341
13. McCluer RH, Gross SK, Sapirstein VS, Meisler MH: Testosterone effects on kidney and urinary glycolipids in the light ear mouse mutant (Abstr). Fed Proc 1979, 38:405
14. Oliver C, Essner E, Zimring A, Haimes H: Age-related accumulation of ceroid-like pigment in mice with Chediak–Higashi Syndrome. Am J Pathol 1976, 84:225–238
15. Peters BP, Goldstein IJ: The use of fluorescein-conjugated *Bandeiraea simplicifolia* B_4-isolectin as a histochemical reagent for the detection of α-D-galactospyranosyl groups. Exp Cell Res 1979, 120:321–334
16. Piccini AE, Jahreis, GP, Novak EK, Swank RT: Intracellular distribution of lysosomal enzymes in the mouse pigment mutants pale ear and pallid. Mol Cell Biochem 1980, 31:89–95

Acknowledgments

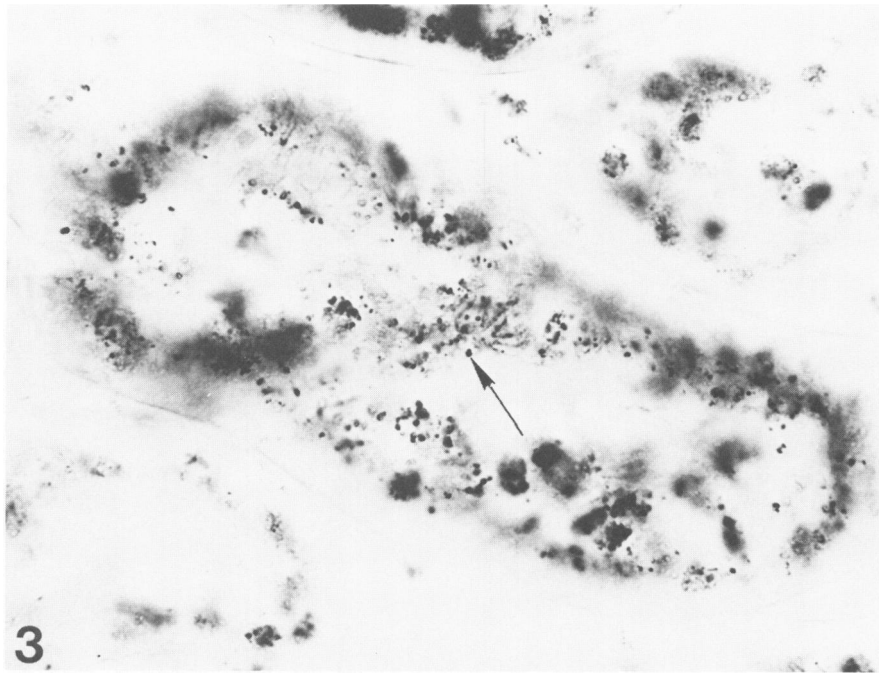
The fluorescence micrograph in Figure 9 was kindly provided by Dr. Barry Peters of the Department of Biological Chemistry, University of Michigan. Sue Plummer assisted with the assay of Cathepsin C.

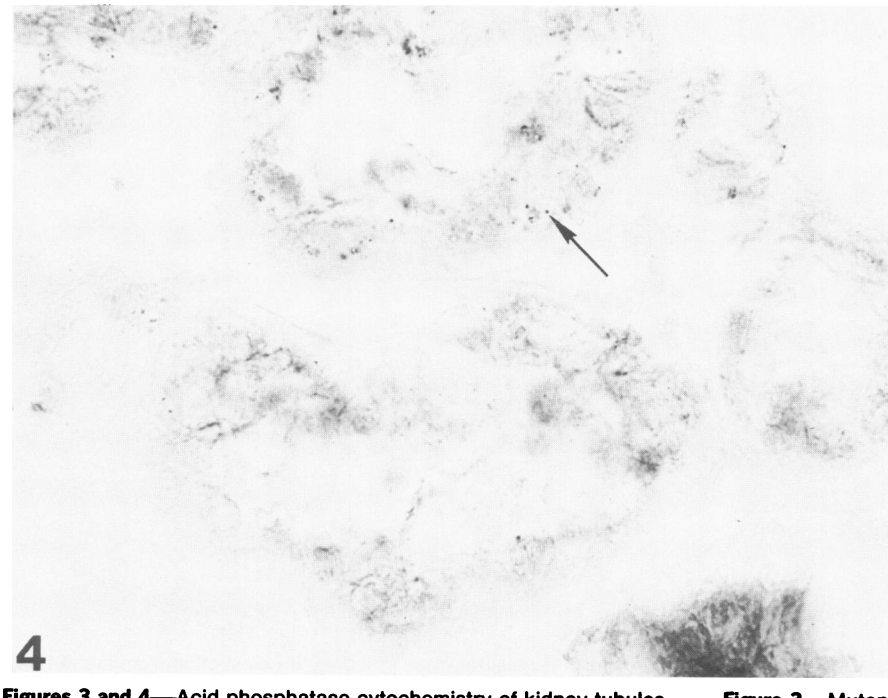
[*Illustrations follow*]



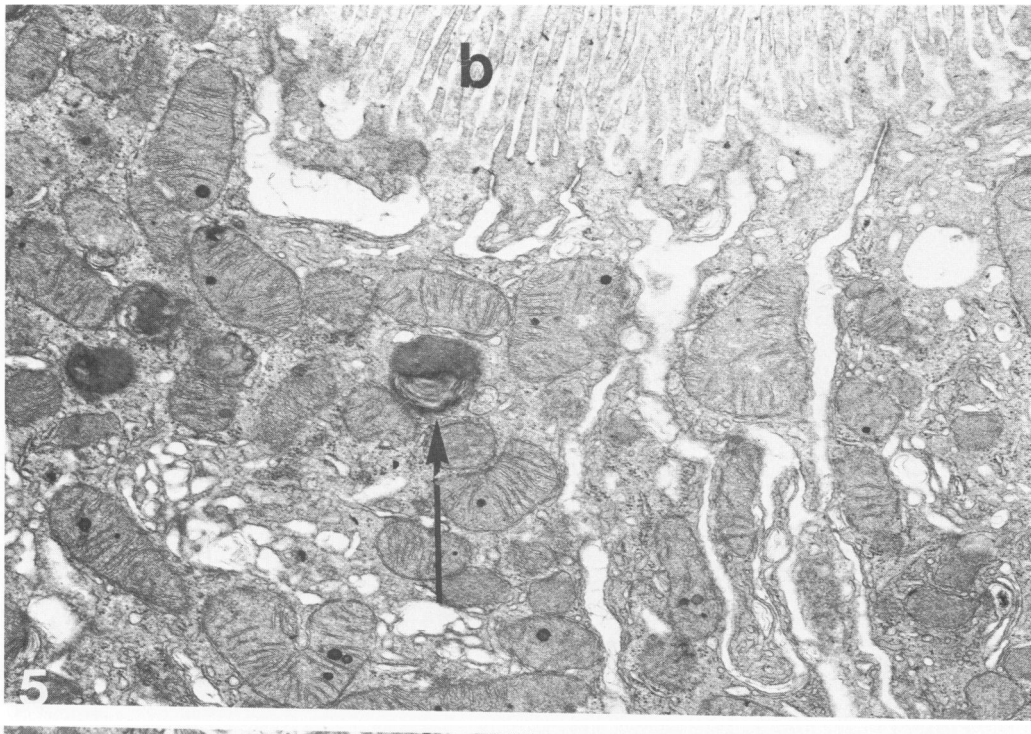


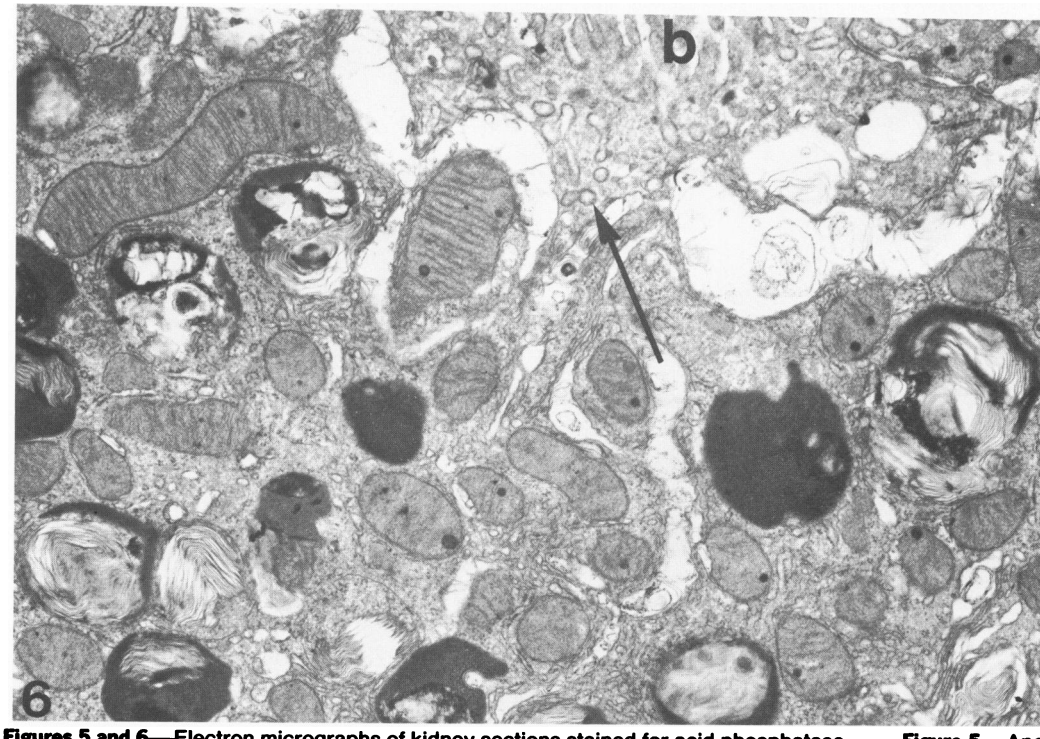
Figures 1 and 2—Histochemical staining of β-galactosidase in kidney cross-section from mutant and control mice. Frozen sections were incubated at 37 C for 30 minutes with 5-bromo-4-chloro-3-indoyl-β-D-galactoside. **Figure 1**—*le/le*. No reaction is seen in the medulla (*M*). Dark areas (*arrow*) are regions of the proximal convoluted tubules. (×14) **Figure 2**—+/+ kidney. Activity is much lower than in the mutant. (×12)



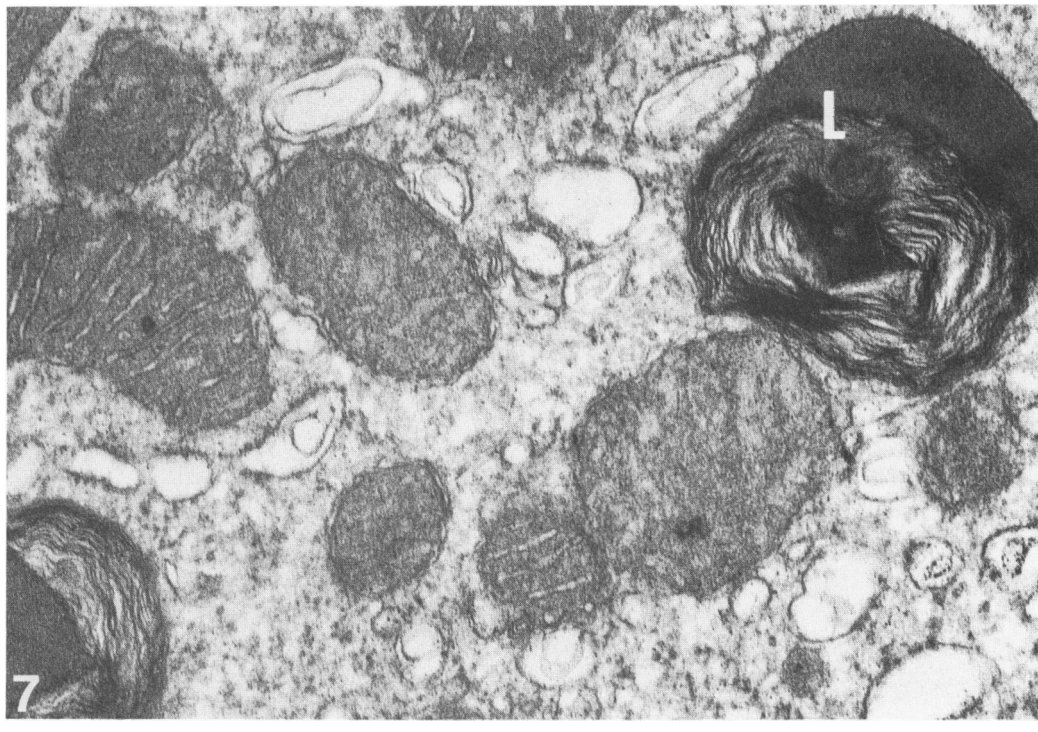


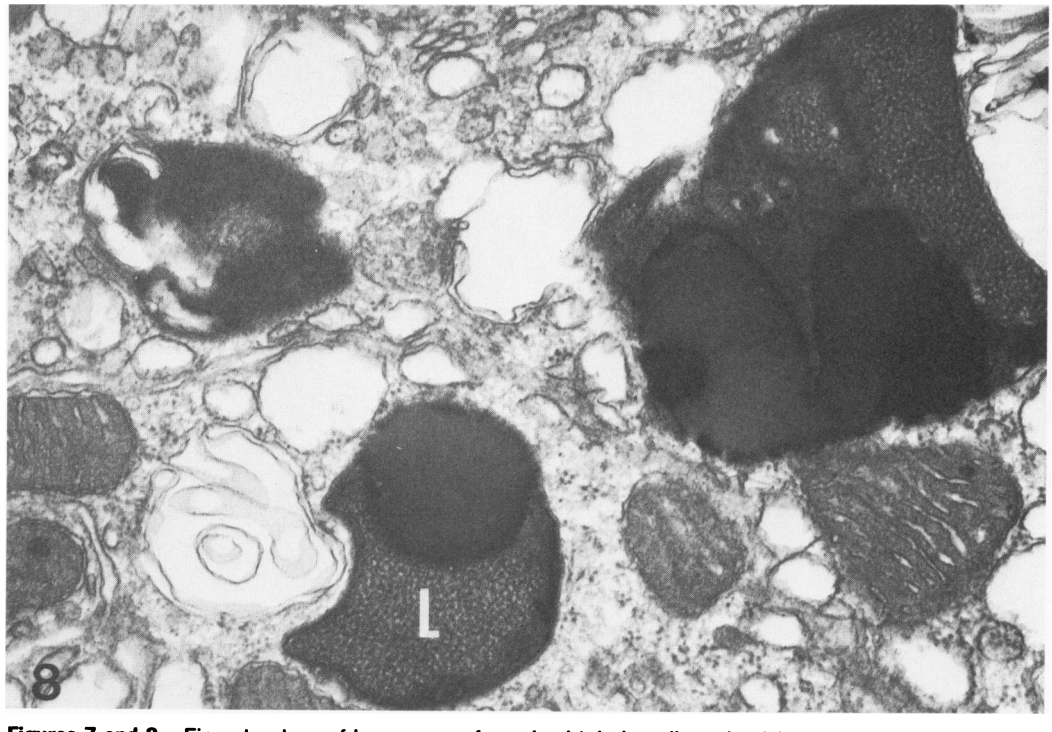
Figures 3 and 4—Acid phosphatase cytochemistry of kidney tubules. **Figure 3**—Mutant le/le kidney section showing numerous large lysosomes (*arrow*) aggregated in proximal convoluted tubule cells. (×1000) **Figure 4**—Lysosomes (*arrow*) are in cells of proximal tubules. Lysosomes are fewer in number and individually much smaller than those seen in the *le/le* strain. (×1000)





Figures 5 and 6—Electron micrographs of kidney sections stained for acid phosphatase. **Figure 5**—Apex of proximal convoluted tubule cell of +/+ kidney showing brush border (*b*) and a few small lysosomes (*arrow*). (×16,000) **Figure 6**—Proximal convoluted tubule cell of an *le/le* male showing small segment of brush border (*b*), numerous endocytotic channels and vesicles (*arrow*) in apical cytoplasm. Lysosomes are larger, have more reaction product, and are more numerous than in the +/+ tubule. (×16,000)





Figures 7 and 8—Fine structure of lysosomes of proximal tubule cells stained for acid phosphatase activity. **Figure 7**—Lysosomes (*L*) from +/+ kidney demonstrating organelles that are composites of dense material and myelin figures. (×45,000) **Figure 8**—Lysosomes from *le/le* kidney. The organelles are large, and the contents often have a honeycomb appearance (*1*) and contain material of varying density. (×46,000)

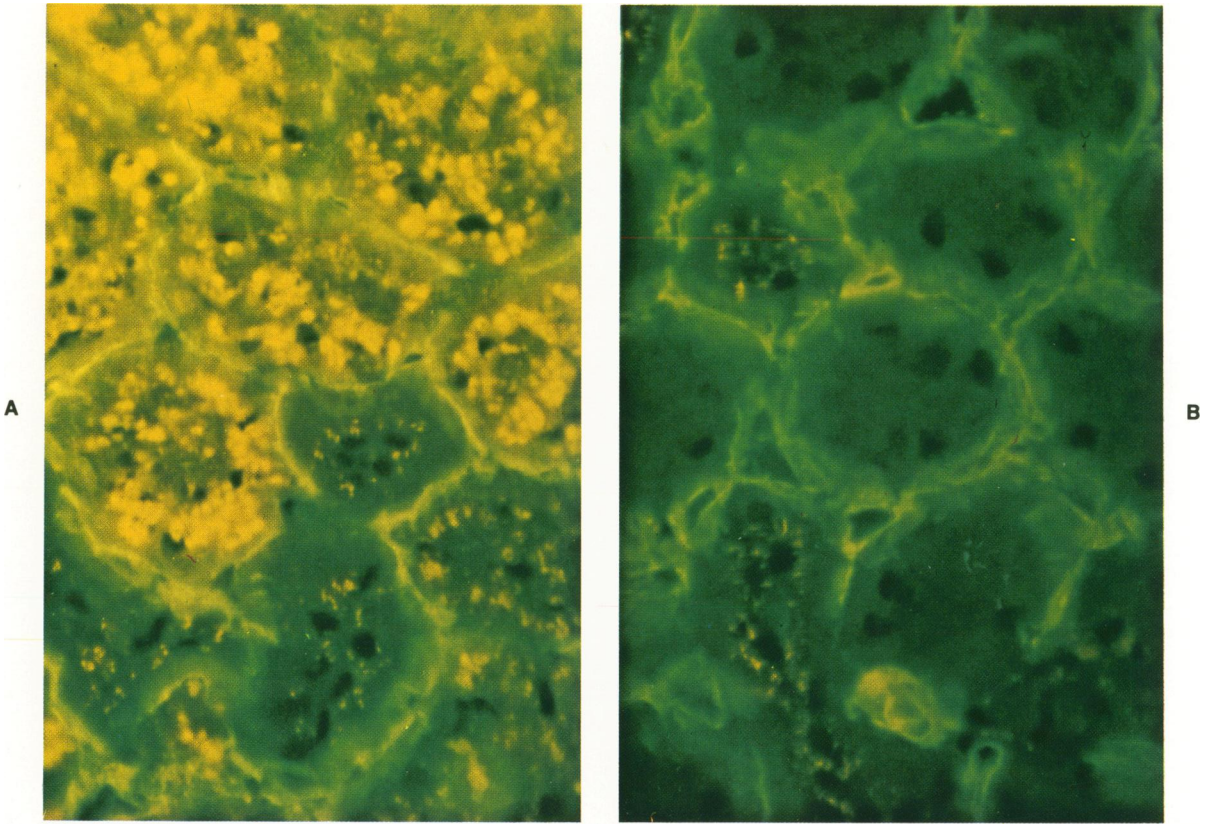


Figure 9—Accumulation of autofluorescent material in proximal tubules of kidney cortex. Frozen sections were illuminated with a 50-watt mercury lamp and examined in a Leitz SM-Lux microscope. **A**—Ie/Ie kidney. **B** —+/+ kidney. (×540)

The cost of publication of this page was defrayed in part by the payment of an illustration charge. This page must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.