Induction of Anomalous Lysosomes in the Renal Papillae of Beige Mice by Experimental Potassium Deficiency (37662)

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The induction of lysosomes in the renal papillae of rats by experimental potassium deficiency is well established (1, 2) and a similar phenomenon in mice has been reported (3). Studies of potassium deficiency have revealed the presence of extensive amounts of new membrane formation within cells of the renal papillae (2). The pathologic effects of potassium deficiency have not been studied in beige mice.

The beige mouse is a homologue of the Chediak-Higashi syndrome (CHS) of man (4). The CHS is an autosomal recessive genetic disease which in addition to man and mice occurs in mink and cattle (5). The CHS is characterized by the presence of large intracellular granules, some of which have been demonstrated to be lysosomes, in many types of cells (6, 7). Thus, this disorder has been referred to as a lysosomal disease (6). The fundamental biochemical defect present in CHS has not been elucidated but several lines of investigation suggest that it is not similar to classical "lysosomal storage diseases" (8, 9). Several investigators have hypothesized that the defect may involve the membrane of the enlarged granules rather than the contents (10, 11). Since both the CHS and experimental potassium deficiency are manifested by apparent alterations in lysosomes and lysosomal membranes, the present study was designed to elucidate the effect of the mutant gene of beige mice on the proliferation of renal papillary lysosomes and their membranes. In normal mice, neither the sequential development nor the morphologic characterization of the lysosomal structures induced by potassium deficiency have been reported previously. Additional investigations were designed therefore to study the sequential development of the lysosomes of the renal papillae in normal mice on a potassium deficient diet, and to compare these findings with those in beige mice and those reported in rats.

Materials and Methods. Young mature male and female beige (bg/bg) mice with the background genes of the C57BL/6J strain and black mice (C57BL/6J) obtained as described previously (12), were used in this study. The experimental design of the study is outlined in Table I. The mice were fasted for 12 hr prior to initiation of the study and were then fed a potassium-free diet (Nutri-

TABLE I. Effects of Potassium Deficiency on the Renal Papillae of Beige and C57 Black Mice.

Group	No. of mice				Diet	Water	Day of sacrifice	Maximal size of lysosomes (μm)	Relative number of lysosomes[a]
	bg ♀	bg ♂	bl ♀	bl ♂					
A	10	10	0	0	K-deficient	deionized	1–10	0–15	0–+[b]
B	0	0	10	10	K-deficient	deionized	1–10	0– 2.7	0–+++[b]
C	2	2	0	0	K-deficient	deionized	26	15	+
D	0	0	2	2	K-deficient	deionized	26	2.7	+++
E	5	5	5	5	K-replenished	tap water	1, 3, 5, 7, 9	0	0
F	2	2	2	2	K-replenished	deionized	5, 10	0	0

[a] 0, +, ++, and +++ refer to none, few, moderate, and many, respectively.
[b] Number increases from 0 to + or +++ with day of sacrifice.

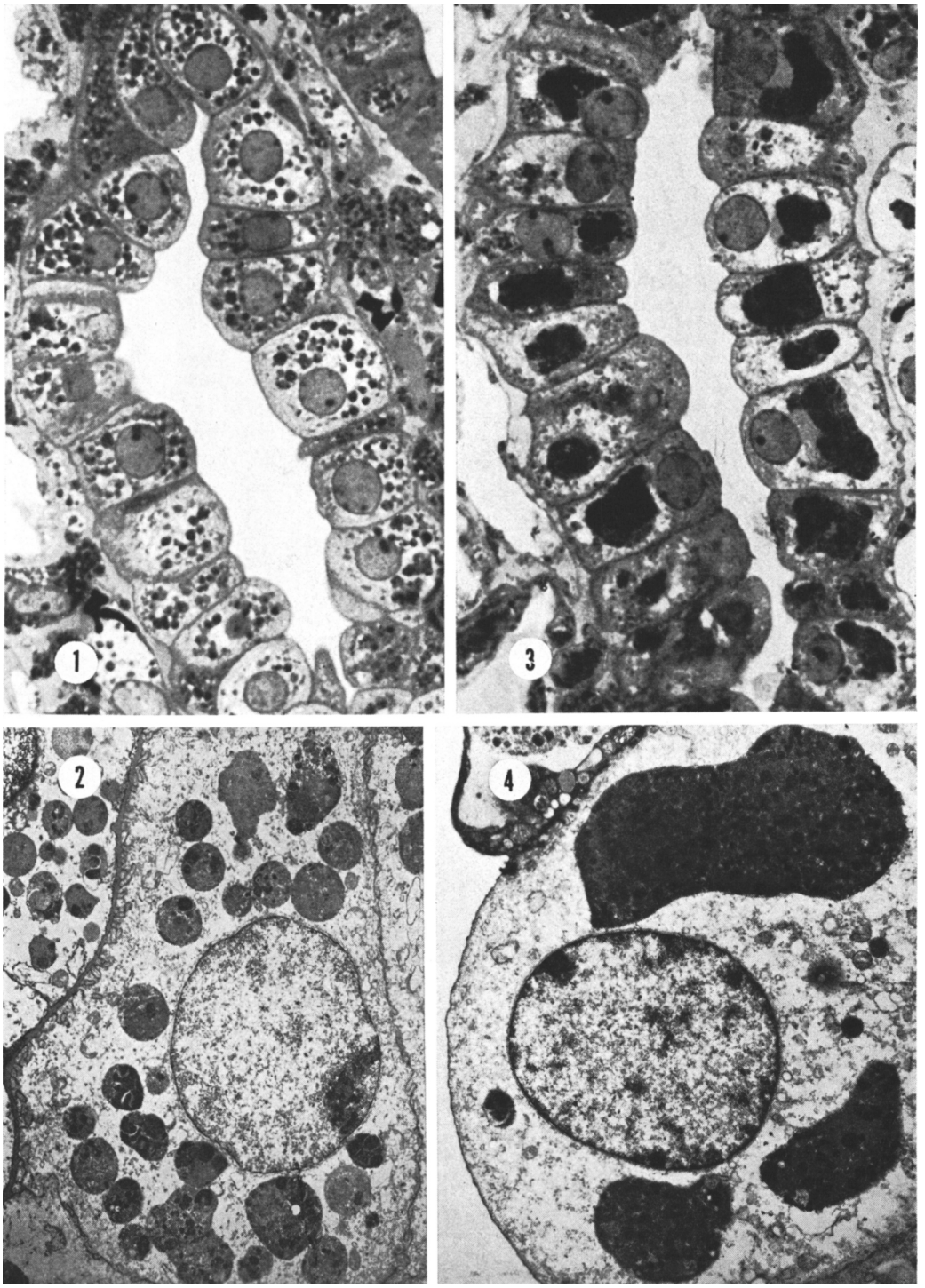

FIG. 1. A 1-μm thick toluidine blue-stained plastic embedded section of a renal papilla of a potassium-deficient normal black mouse. Note the large number of lysosomes in the cells of the tubules. 1,050×.

tional Biochemicals) or a potassium-free diet replenished with 0.8 g of K_2HPO_4 and 0.6 g of KCl/100 g of diet (1). Either deionized or tap water was provided *ad libitum* according to the assigned group in Table I. The mice were kept in plastic cages with sawdust bedding and feed was available *ad libitum* throughout the study. At the designated times, the mice were killed by cervical dislocation and the kidneys were removed. The papilla was removed from one kidney, hemisected longitudinally, and fixed in 1.5% glutaraldehyde in 0.1 *M* potassium phosphate buffer (pH 7.1) and 1% sucrose. In 50% of the mice, half of this papilla was fixed in Carnoy's solution. The other kidney was fixed in 10% neutral buffered formalin. The formalin fixed kidneys were processed, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin and with periodic acid-Schiff (PAS). The Carnoy's fixed papillae were also embedded in paraffin and sectioned at 6 μm but were stained with PAS and with Best's carmine stain with and without diastase digestion. The glutaraldehyde fixed papillae were rinsed with buffer, postfixed in osmium tetroxide, dehydrated in graded ethanols and embedded in Epon-Araldite. Sections approximately 1.0 μm in thickness were stained with 1% heated toluidine blue and examined under oil immersion by light microscopy. Selected areas were sectioned with an ultramicrotome, stained with lead citrate and uranyl acetate, and examined with a Jeolco 100B electron microscope.

Results. Effects of potassium deficiency on normal mice. During the second and third day after being placed on the potassium-deficient diet, the black mice developed PAS positive lysosomes within the cytoplasm of the cells of the renal papillae. These bodies (lysosomes) increased in size and number until approximately the eighth day. The lysosomes were most numerous at the tip of the papillae and less numerous in cells more closely located toward the medulla proper. Within one day after the appearance of the bodies in the cells of the collecting tubules, they could be observed in interstitial and endothelial cells of the renal papillae. The lysosomes were not as numerous in these types of cells, however, as they were in cells of the tubules. The number and size of lysosomes in the cells did not appear to increase appreciably between 8 and 26 days after the initiation of feeding of the potassium-free diet.

No difference was observed in the development of the bodies in male or female mice. The lysosomes did not develop in mice fed a potassium-replenished diet and provided with tap water or deionized water. By light microscopy the bodies appeared round and hyalinized, staining eosinophilic with H & E stain. They were intensely positive with PAS stain and retained PAS positivity after diastase digestion. They were negative for glycogen with Best's carmine stain. Examination of the toluidine blue-stained plastic embedded sections revealed large numbers of dense bodies in the cytoplasm of the cells of the renal papillae (Fig. 1). Ultrastructurally, the lysosomes consisted of a heterogeneous accumulation of vesicular structures and dense granules. The bodies varied in size up to 2.7 μm in diameter (Fig. 2). Most were round to oval in cross section and were limited by a trilaminar and occasionally discontinuous membrane. The bodies were randomly distributed in both the apical and basilar portions of the epithelial cells. The lysosomes were composed of three primary constituents: 1) whorls of membraneous material, 2) small, moderately dense granules, approximately 400 Å in diameter, and 3) large, very dense gran-

FIG. 2. Electron micrograph of a renal papillary epithelial cell from 9-day potassium-deficient black mouse. Note the number and size of the lysosomes compared to Fig. 4. Uranyl acetate and lead citrate. 5,000×.

FIG. 3. A 1-μm thick toluidine blue-stained plastic embedded section of a renal papilla of a potassium-deficient beige mouse. There is an aggregation of the lysosomes into large masses which, in some of the cells, displace the nuclei from the cell centers. Compare with Fig. 1. 1,050×.

FIG. 4. Electron micrograph of a renal papillary epithelial cell from a 9-day potassium-deficient beige mouse. Note the 3 large lysosomes. By serial sectioning lysosomes similar to these were shown to be continuous. Uranyl acetate and lead citrate. 5,000×.

ules, approximately 0.25–0.40 μm in diameter, occasionally partially enveloped by a membraneous component. These larger granules appeared to be composed of condensed aggregations of the smaller granules. The three components of the bodies were randomly distributed within individual lysosomes without apparent fixed proportions except that the membraneous whorls appeared to be more predominant in lysosomes during the first days of potassium deficiency.

Effects of potassium deficiency on beige mice. The temporal relationships of the development of renal papillary lysosomes due to potassium deficiency was the same in the beige mice as that described in the black mice. In addition, no difference was observed in the area of the kidney nor cell types involved in the induced changes in the two strains of mice. A striking difference was noted, however, in the size of the induced granules. By light microscopy, PAS positive crescent shaped granules varying in size up to 15 μm in length and approximately 8 μm in width were observed. Examination of plastic-embedded toluidine blue-stained sections of renal papillae revealed large aggregates of granules up to 15 μm in length (Fig. 3). These large lysosomes, some having a crescent shape, appeared to be composed of a heterogeneous accumulation of small dense granules. In many of the tubular cells they displaced the nucleus from the central area of the cell (Fig. 3). By electron microscopy it was observed that the lysosomes were bounded by an incomplete unit membrane and were composed of whorls of membraneous material, and small and large dense granules indistinguishable in structure from those observed in the black mice. These large lysosomes were observed occasionally to have a crescent shape ultrastructurally but more often there were several large lysosomes which appeared to be discrete because of the plane of the sections (Fig. 4). In addition to the large bodies there were also a few small lysosomes in the cytoplasm of the cells similar in size and structure to those observed in the black mice.

No difference could be discerned in the total amount of material accumulated into lysosomes in renal papillary cells in the two strains of mice.

Discussion. The morphologic appearance of the lysosomes in the black mice was very similar to that demonstrated in rats at both the light and ultrastrucural levels. The total number of lysosomes per cell in the mice appeared less, however, than those demonstrated in rats by Wilson and coworkers (2). More recently they also demonstrated a linear increase in the phospholipid content of the inner renal medulla for at least 20 days after the initiation of a potassium-deficient diet although no mention is made of a corresponding increase in the number of lysosomes (13). In the present study there was no demonstrable difference in the number or appearance of lysosomes in mice between 8 and 26 days of potassium deficiency. In this study, however, the mice were kept on sawdust bedding and may therefore have been able to stabilize their potassium at higher levels by coprophagy and bedding ingestion. On the other hand, this may represent an actual difference between the two species.

The lysosomes that developed in the beige mice with potassium deficiency were larger, but the internal structure was similar to those in the normal mice. This observation along with the smaller number of granules in the beige mice suggests a process of fusion as the mechanism by which the larger lysosomes were formed. This has been the suggested operative mechanism of enlarged granule formation in other types of cells in the CHS. The giant melanosomes in the skin of patients with CHS have been shown to be a result of the excessive fusion of premelanosomes (14). The abnormally enlarged granules in the polymorphonuclear leukocytes of mink with CHS have been shown to arise from an excessive continued fusion of the primary granules (9). The secondary granules in these leukocytes, however, were not involved in the fusion process and remained normal (9).

In the present study the substructure of the enlarged renal lysosomes in the potassium deficient beige mice contained numerous membraneous vesicles, whorls, and partially membrane enveloped dense granules which had no evidence of fusion. This suggests that the outer membrane of these lysosomes may be genetically distinct from the membraneous

material within the lysosomes. Although hypotheses have been proposed implicating a defect in the membranes of the enlarged lysosomes in CHS (10, 11), the only experimental evidence to support these hypotheses is an accelerated turnover rate of membrane lipids in this disorder (15).

No enlarged lysosomes were observed in renal papillae of beige mice which were not potassium deficient. This study illustrates the limitations of implying that a certain tissue or type of cell is not abnormal in CHS. It may be possible to induce the abnormal lysosomal structures in other apparently normal cells if the proper stimulus, *e.g.*, potassium deficiency, is applied.

Summary. Normal mice which were fed a potassium-deficient diet and deionized water developed large numbers of lysosomes in the cytoplasm of the cells of the renal medulla similar in size, structure, and distribution upon light and electron microscopic examination to those induced in rats by the same dietary manipulations. However, beige mice, which are homologues for the Chediak-Higashi syndrome, developed a smaller number of much larger lysosomes which were structurally similar to the lysosomes in potassium-deficient normal mice. It is concluded that the large lysosomes in the renal papillae of beige mice originated by fusion of smaller lysosomes and that the smaller lysosomes arose by the same mechanism as the lysosomes in the potassium-deficient normal mice.

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