Effect of Potassium Deficiency on Mouse Kidney Lysosomal Enzymes

By CAROLYN E. CLEVELAND and RICHARD T. SWANK

Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, NY 14263, U.S.A.

(Received 23 June 1977)

Mice of inbred strains A/J, C57BL/6J and C57BL/6J beige were kept on a K⁺-deficient diet for up to 40 days to determine the magnitude and mechanism of changes in tissue lysosomal enzymes. From days 10 to 40 glucuronidase activity increased 3-fold in kidney of K⁺-deficient mice, but there was little effect on β -galactosidase or acid phosphatase activity. Similar increases in kidney glucuronidase activity occurred in inbred strains known to have genetically altered control of the synthesis (A/J) and secretion (C57BL/6J beige) of glucuronidase in kidney proximal-tubule cells. Deprivation of K⁺ did not affect glucuronidase activity in liver, spleen, lung and brain, but there was a 2–3-fold increase in glucuronidase activity in heart in the C57BL/6J and C57BL/6J beige strains. As shown by specific antibody titration, increased glucuronidase activity in kidney of K⁺-deficient mice was accompanied by accumulation of enzyme molecules. Likewise in kidney of deficient mice there was an increased rate of synthesis of glucuronidase as measured by incorporation of labelled leucine into immunoprecipitable glucuronidase. In kidney of K⁺-deficient mice the elevated glucuronidase activity was found in both collecting-tubule and interstitial cells of the medulla. It is probable therefore that a significant fraction of the increased kidney lysosomal synthesis and enzyme activity is due to infiltrating cells.

When rats are fed on a diet deficient in K⁺, there is a 6-fold increase in acid phosphatase activity in cells of the kidney medulla (Morrison & Panner, 1964), and 2–4-fold increase in four other lysosomal enzymes (Aithal *et al.*, 1977). Within a few days these cells accumulate dense granules which stain intensely with the periodic acid/Schiff reagent and contain cytochemically identifiable acid phosphatase activity (Morrison & Panner, 1964; Muehrcke & Rosen, 1964). The kidney of K⁺-deficient rats has been proposed therefore as a model system to study biogenesis of lysosomes in mammals.

Much of the increase in lysosome content occurs in collecting-tubule cells of the renal papilla. There is also an increase in numbers of dense granules in collecting-tubule cells throughout the medulla and to a lesser extent in interstitial and endothelial cells of the medulla (Spargo *et al.*, 1960; Morrison & Panner, 1964; Toback *et al.*, 1976). However, the molecular mechanisms modulating the increased activities of lysosomal enzymes remain unknown.

Other physiological effects of K⁺ deficiency include growth retardation and inability to concentrate the urine (Manitius *et al.*, 1960). In the kidney medulla there is a considerable increase in both cell size and DNA content accompanied by increases in the synthesis of protein, RNA, DNA (Gustafson *et al.*, 1973) and phospholipids (Wilson *et al.*, 1973).

There have been few studies of the effects of K⁺ deprivation in the mouse. Liebow *et al.* (1941) found effects on kidney similar to those in the rat, including hypertrophy and proliferation of medullary-tubule cells. Prieur *et al.* (1973) studied the accumulation of cytochemically detectable granules, probably lysosomes, in kidney of K⁺-deficient normal and beige mice. The beige mouse is an animal model for the human Chediak-Higashi syndrome and has giant lysosomes in many tissues (Oliver & Essner, 1973). In both types of mice K⁺ deprivation caused an increase in lysosomes of cells of the medulla, the increase occurring first in cells of the collecting tubules, then in interstitial and endothelial cells. In cells of the beige mouse, giant lysosomes, up to 15 μ m in diameter, accumulated.

There are advantages to studying lysosomal biogenesis in the mouse, including the facts that different inbred strains contain mutant genes that affect lysosomal enzymes in several respects, including their structure, rate of synthesis, temporal appearance during development and processing (Paigen *et al.*, 1975). In the present study we have examined the effects of K⁺ deficiency on the inbred strains C57BL/6J, A/J and C57BL/6J beige. Strain A/J has a mutant allele *Gur*^a, which increases the rate of synthesis of β -glucuronidase in kidney proximal-tubule cells of androgen-treated female mice to three times that of similarly treated C57BL/6J mice (Swank *et al.*, 1973). The beige gene in C57BL/6J beige mice increases the activities of all lysosomal enzymes in

Abbreviation used: SDS, sodium dodecyl sulphate.

kidney proximal-tubule cells by greatly slowing the normal secretion of these enzymes into urine (Brandt *et al.*, 1975).

The purpose of these experiments was to determine if the K^+ -deficient mouse is a favourable system for the study of lysosomal biogenesis. Since glucuronidase activity was found to be significantly elevated in the kidney of K^+ -deficient mice, we explored (a) the cellular and molecular basis of the increased enzyme activity and (b) the possible effect on this experimental system of mutations in the mouse that are known to affect synthesis and secretion of lysosomal enzymes.

Materials and Methods

Animals

Female A/J, C57BL/6J and C57BL/6J beige-J (bg^1/bg^1) mice, 8–16 weeks old, were obtained from the Jackson Laboratory, Bar Harbor, ME, U.S.A. All mice were kept on a 12h-light/12h-dark schedule. Experimental mice were fed *ad libitum* on a K^+ -deficient diet together with deionized water. K^+ -replenished mice were given tap water, and fed on K^+ -deficient diet supplemented with 0.8 g of K_2HPO_4 and 0.6 g of KCl/100 g of K^+ -deficient diet. Cages were changed every other day to prevent coprophagy. Plasma K^+ in 30-day-deficient mice ranged from 1.9 to 2.9 mequiv./litre, compared with 4.1–4.8 mequiv./litre in replenished animals. Animals were killed by either cervical dislocation or by anoxia with CO_2 gas. Excised tissues were weighed and then homogenized for 1 min in approx. 10 vol. (w/v) of ice-cold 0.25 M-sucrose containing 0.02 M-imidazole buffer adjusted to pH 7.4 with HCl, with a 12 mm Polytron homogenizer (Kinematica G.m.b.H., Lucerne, Switzerland) at setting 4.8. Homogenates were stored at $-20^\circ C$.

Enzyme assays

β -Glucuronidase (EC 3.2.1.31) and β -galactosidase (3.2.1.23) were assayed by the fluorimetric procedures described by Brandt *et al.* (1975) with 4-methylumbelliferyl β -glucuronide and 4-methylumbelliferyl β -D-galactoside respectively as substrates. Acid phosphatase (3.1.3.2) was measured by the modification described below (R. Elliott, personal communication) of the method of Hers *et al.* (1951), with 0.05 M- β -glycerophosphoric acid as substrate. Appropriately diluted homogenates were assayed at $37^\circ C$ for 20–40 min in 0.046 M-sodium acetate adjusted to pH 5.2 with acetic acid. The reaction was terminated by adding trichloroacetic acid to 4% (w/v), and reaction tubes were centrifuged for 10 min at 1600g. Phosphate was determined in 1 ml of supernatant by adding 0.25 ml of 6% (w/v) ammonium molybdate in 20% (v/v) H_2SO_4 , mixing, then adding 0.05 ml of 1% Elon (Eastman Kodak,

Rochester, NY, U.S.A.) in 10% (w/v) sodium metabisulphite. This mixture was incubated for 15 min at $37^\circ C$. The A_{800} of experimental solutions and solutions of standard KH_2PO_4 were determined after this treatment. For all enzyme assays unit of activity is defined as the amount of enzyme required to form $1 \mu mol$ of product/h at $37^\circ C$. All enzyme assays were performed under conditions where product formation was proportional to time of incubation and to homogenate concentration.

Immunotitration

Kidney glucuronidase activity, which is very low in female mice, was titrated with antibody by the method of R. Davey (personal communication) as described below. Increasing amounts of goat anti-(mouse glucuronidase) immunoglobulin in 0.15 M-NaCl/0.02 M-Tris adjusted to pH 7.4 with HCl were added to kidney homogenates containing approx. 0.05 unit of glucuronidase activity. Approx. $30 \mu g$ of goat carrier immunoglobulin fraction was added and samples were incubated at $37^\circ C$ for 30 min and left for 24 h at $4^\circ C$. Sufficient rabbit anti-(goat antibody) was added to precipitate the goat immunoglobulin completely. The samples were then incubated for 30 min at $37^\circ C$ and kept at $4^\circ C$ overnight. Immunoprecipitates were collected by centrifugation at 16000g for 10 min and washed once with 0.5 ml of 0.15 M-NaCl in 0.2 M-Tris adjusted to pH 7.4 with HCl. Pellets were finally resuspended in 0.2 M-sodium acetate adjusted to pH 4.6 with acetic acid and containing 0.2% (v/v) Triton X-100. Glucuronidase activity was determined in each immunoprecipitate and plotted as a function of amount of goat anti-(mouse glucuronidase) added. The slope of the resulting line is a sensitive measure of the immunoreactivity of the sample glucuronidase.

Rates of synthesis of glucuronidase

Each mouse received $300 \mu Ci$ of L-[4,5- 3H]leucine intraperitoneally and was killed 1 h later. Kidney homogenates from three mice were pooled and rates of synthesis of glucuronidase measured by specific antibodyprecipitation of heat-treated and $(NH_4)_2SO_4$ -fractionated homogenate as described by Brandt & Swank (1976), with the following modifications.

Before immunoprecipitation total glucuronidase activity of the homogenate was adjusted to 20 units by addition of a glucuronidase fraction partially purified from urine of mice treated with testosterone for 15–40 days. Also, non-specific contaminants in the immunoprecipitate were removed by electrophoresis on SDS/polyacrylamide gels. Samples were boiled for 2 min in 0.08 ml of 2% (w/v) SDS/24% (w/v) urea/5% (v/v) mercaptoethanol and electrophoresed in 6 mm-diameter 10% polyacrylamide gels at 3 mA/gel until a Bromophenol Blue dye marker had migrated

90 mm (Laemmli, 1970). Gels were sliced into 1.25 mm sections, and swollen overnight at room temperature (25°C) in NCS/water (9:1, v/v). To each sample was added 10 ml of Scintiprep 2 toluene scintillant (Fisher Scientific Co., Fair Lawn, NJ, U.S.A.). Samples were counted for radioactivity in a Nuclear-Chicago Isocap 300 liquid-scintillation counter with 36% efficiency and a background of 17 c.p.m. Gel slices in the glucuronidase peak were counted for sufficient time to give less than 10% error. Counts were corrected for an observed 90% (83–93%) recovery of the counts applied to a gel in the sum of the gel fractions.

Radioactivity incorporated into total cell protein was determined by spotting 10 μ l of total kidney homogenate on to 2.1 cm Whatman filter-paper discs. The discs were boiled for 15 min in 5% (w/v) trichloroacetic acid and were rinsed in 5% trichloroacetic acid containing 0.05 M-L-leucine and then in ethanol/ether (1:1, v/v). After air-drying the discs were added to scintillation vials containing 1 ml of NCS/water (9:1, v/v) and incubated for 1.5 h at 50°C before addition of scintillant. The rate of synthesis of glucuronidase is defined as the ratio of radioactivity incorporated into the 73000-mol.wt. glucuronidase subunit peak on the polyacrylamide gel to that incorporated into total acid-precipitable protein. Radioactivity incorporated into glucuronidase was corrected for loss of enzyme activity between preparation of the original homogenate and the antibody-precipitation step. In these experiments comparable yields of glucuronidase activity, between 67% and 78%, were obtained in both normal and K⁺-deficient mice.

Histology

Kidneys were removed, decapsulated and fixed in CaCl₂/formalin as previously described (Brandt *et al.*, 1975). Sections 8 μ m thick were stained for 2 h for glucuronidase activity by using the naphthol AS-BI β -D-glucuronide/hexazonium/pararosanilin method described by Hayashi (1964). The sections were counterstained with 0.1% Methyl Green (Eastman

Organic Chemicals, Rochester, NY, U.S.A.) in 0.1 M-sodium citrate, pH 4. The specificity of the glucuronidase-staining method was verified by using saccharolactone, a potent glucuronidase inhibitor, at 0.125 mM final concentration in the Naphthol AS-BI β -D-glucuronide substrate solution.

Reagents and materials

K⁺-deficient diet, completely fortified with vitamins and with sucrose replacing starch, was obtained from ICN Pharmaceuticals, Cleveland, OH, U.S.A. 4-Methylumbelliferyl galactoside was provided by Research Products International Corp., Elk Grove, IL, U.S.A. Naphthol AS-BI β -D-glucuronide and 4-methylumbelliferyl β -D-glucuronide were from Sigma Chemical Co., St. Louis, MO, U.S.A. β -Glycerophosphoric acid was from Eastman Organic Chemicals. NCS solubilizer and L-[4,5-³H]leucine (50 Ci/mmol) were supplied by Amersham/Searle, Arlington Heights, IL, U.S.A. Goat anti-(mouse glucuronidase) immunoglobulin was kindly supplied by Dr. Shiro Tomino, Tokyo Metropolitan University, Tokyo, Japan. Rabbit anti-(goat immunoglobulin) was obtained from Miles Laboratories, Kankakee, IL, U.S.A. All chemicals were reagent grade.

Results

Glucuronidase in organs of K⁺-deficient mice

Two organs, kidney and heart, among the six organs surveyed (Table 1), had altered glucuronidase activity after mice were deprived of K⁺ for 30 days. Kidney glucuronidase activity increased 2–3-fold ($P < 0.001$ by Student's *t* test) in K⁺-deficient A/J, C57BL/6J and C57BL/6J beige strains. The increase in total kidney glucuronidase in K⁺-deficient mice was actually considerably more than indicated by the data of Table 1 since, owing to hypertrophy of medullary cells, kidneys of K⁺-deficient mice were 30–40% heavier than kidneys of K⁺-replenished mice after the 30 days of this experiment. Only the two C57BL/6J strains contained increased glucuronidase

Table 1. Glucuronidase activity in organs from K⁺-replenished (+) and K⁺-deficient (–) mice
Values are means $\pm$ S.E.M. for three mice killed after 30 days on the indicated diet.

Strain	Dietary K ⁺	Glucuronidase activity (units/g)					
		Kidney	Heart	Liver	Spleen	Lung	Brain
A/J	+	8.3 $\pm$ 0.38	1.01 $\pm$ 0.08	19.6 $\pm$ 2.01	46.1 $\pm$ 4.6	5.6 $\pm$ 0.4	1.3 $\pm$ 0.05
A/J	–	17.3 $\pm$ 0.33	1.2 $\pm$ 0.02	19.9 $\pm$ 0.39	47.4 $\pm$ 3.2	9.0 $\pm$ 2.0	1.5 $\pm$ 0.08
C57BL/6J	+	9.3 $\pm$ 0.26	0.85 $\pm$ 0.04	24.2 $\pm$ 1.10	38.8 $\pm$ 2.9	6.8 $\pm$ 0.6	1.32 $\pm$ 0.09
C57BL/6J	–	22.0 $\pm$ 0.18	1.90 $\pm$ 0.14	27.1 $\pm$ 0.15	37.6 $\pm$ 2.1	8.1 $\pm$ 1.6	1.18 $\pm$ 0.05
C57BL/6J (<i>bg</i> ⁺ / <i>bg</i> ⁺)	+	11.5 $\pm$ 0.53	0.80 $\pm$ 0.04	23.7 $\pm$ 1.30	29.8 $\pm$ 2.7	5.6 $\pm$ 0.6	1.31 $\pm$ 0.27
C57BL/6J (<i>bg</i> ⁺ / <i>bg</i> ⁺)	–	26.7 $\pm$ 0.80	2.3 $\pm$ 0.41	19.6 $\pm$ 1.81	35.0 $\pm$ 0.9	5.2 $\pm$ 0.4	1.34 $\pm$ 0.04

in heart, and, as in kidney, the increase in heart glucuronidase was 2–3-fold ($P < 0.05$). K^+ deprivation did not significantly affect the fresh weight of the heart or the other organs. There was little interstrain variation in glucuronidase activities in organs. However, kidney glucuronidase activity was 20–25% higher ($P < 0.05$) in C57BL/6J beige mice than in the two normal strains whether K^+ -replenished or K^+ -deficient mice were examined.

There was no change in kidney glucuronidase activity before 10 days of K^+ deprivation (Fig. 1). From day 10 until day 40, however, activity accumulated at a near-linear rate in all three strains. Kidney glucuronidase activity in strain C57BL/6J was about 20% higher than in strain A/J at days 20–40. In turn, activity in kidney of the C57BL/6J beige strain was about 25% higher than in the congenic C57BL/6J normal strain at all time points, including day 0, before K^+ deprivation.

Fig. 1. Time course of accumulation of glucuronidase activity in kidney of K^+ -deficient mice

Strains: ●, C57BL/6J beige; ○, C57BL/6J; △, A/J. Each point is the mean value for three mice.

Other lysosomal enzymes

When two other lysosomal hydrolases, β -galactosidase and acid phosphatase, were assayed in kidney homogenates of 30-day-replenished and K^+ -deficient mice, no significant effect ($P > 0.05$) of K^+ deficiency on the activities of these enzymes within any inbred strain was noted (Table 2). Likewise, no strain differences in activities of these enzymes were apparent. There was, in fact, no significant difference ($P > 0.2$) in galactosidase activities even when the mean activity (26.7 units/g) of the kidneys of K^+ -replenished mice of all three strains was compared with the mean activity (30.8 units/g) of the kidneys of K^+ -deficient mice of all three strains. However, a similar calculation revealed that the mean acid phosphatase activity (156 units/g) in kidneys of K^+ -deficient mice of all three strains was 30% higher ($P < 0.05$) than the mean acid phosphatase activity (121 units/g) in kidneys of K^+ -replenished mice.

Mechanism of elevation of kidney glucuronidase activity

Two experiments suggested that the increased glucuronidase activity in kidney of K^+ -deficient mice was due to accumulation of new enzyme molecules rather than to activation of pre-existing molecules or formation of a new species of enzyme with glucuronidase activity. First, glucuronidase activity in mixtures of kidney homogenates from K^+ -deficient and K^+ -replenished mice was additive (results not shown). Second, immunotitration (Fig. 2) of glucuronidase activity in kidney homogenates of K^+ -deficient and K^+ -replenished A/J mice resulted in precipitation of equal amounts of activity per unit of anti-glucuronidase antibody added. Likewise, no significant differences in reactivity with antibody were found when kidney extracts from K^+ -deficient and K^+ -replenished C57BL/6J or C57BL/6J beige mice were compared.

Table 2. Activities of lysosomal enzymes in kidney of K^+ -replenished (+) and K^+ -deficient (–) mice
Values are means $\pm$ S.E.M. for three mice killed after 30 days on the indicated potassium diet.

Strain	Dietary K^+	Enzyme activity (units/g of kidney)	
		β -Galactosidase	Acid phosphatase
A/J	+	26.9 $\pm$ 2.1	108 $\pm$ 8.8
A/J	–	35.8 $\pm$ 3.2	139 $\pm$ 4.3
C57BL/6J	+	26.5 $\pm$ 2.0	123 $\pm$ 8.0
C57BL/6J	–	32.8 $\pm$ 2.7	150 $\pm$ 10
C57BL/6J (<i>bg¹/bg¹</i>)	+	27.8 $\pm$ 0.8	133 $\pm$ 8.2
C57BL/6J (<i>bg¹/bg¹</i>)	–	24.7 $\pm$ 2.3	185 $\pm$ 14

Fig. 2. Immunotitration of glucuronidase activity in kidney extracts of K^+ -deficient (Δ) and K^+ -replenished ($\blacktriangle$) C57BL/6J mice

Glucuronidase activity was measured in the immunoprecipitate after addition of increasing amounts of antibody to 0.05 unit of glucuronidase in solution.

We next tested whether the increased accumulation of glucuronidase molecules in kidneys of K^+ -deficient mice was due to a more rapid enzyme synthesis. When glucuronidase-antibody precipitates from 3H -labelled C57BL/6J mice were electrophoresed on denaturing polyacrylamide gels (Fig. 3), a distinct peak of 3H was observed for both K^+ -deficient and K^+ -replenished mice at a position in the gel corresponding to the electrophoretic mobility of the 73000-mol.wt. glucuronidase subunit. Total radioactivity in the glucuronidase subunit was 2–3 times greater in 22-day- K^+ -deficient mice than in K^+ -replenished mice when antibody precipitates prepared from equivalent amounts of kidney extracts were compared. Purification of the antibody precipitate on SDS/polyacrylamide gels was necessary because of the extremely low rate of synthesis of kidney glucuronidase (about 0.001% of total kidney protein synthesis). This rate is in fact 100-fold lower than that in kidneys from androgen-treated mice (Swank *et al.*, 1973), where non-specific contaminants are an insignificant proportion of the total antibody-precipitable radioactivity. Most of

the non-specific contaminants in the glucuronidase immunoprecipitate in these experiments are of low molecular weight and can be greatly decreased by isolating glucuronidase with anti-glucuronidase immunoglobulin covalently coupled to a Sepharose column (G. Watson, personal communication).

Increased kidney glucuronidase activity attendant on K^+ depletion is paralleled by an increased rate of synthesis of this lysosomal enzyme (Table 3). At day 15 the relative rate of glucuronidase synthesis was in K^+ -deficient mice 1.5-fold ($P < 0.05$) that in K^+ -replenished mice. By day 22 the increase was 1.9-fold ($P < 0.02$). At day 30 the relative rate of synthesis in deficient mice was about 1.4-fold ($P < 0.05$) that of replenished mice. There was an apparent increase in radioactivity incorporated into glucuronidase and total protein in 30-day- K^+ -deficient mice compared with 22-day- K^+ -deficient mice, but most of this increase was due to the fact that these animals weighed about 30% less than the 15- or 22-day-deficient animals, with a consequent decrease in dilution of injected radioisotope. Finally, the radioactivity incorporated into glucuronidase per g of kidney is a slight underestimate of total kidney glucuronidase synthesis in all K^+ -deficient mice, since the kidneys of K^+ -deficient mice in these experiments were 14–25% larger than those of K^+ -replenished mice.

Cellular site of increased kidney glucuronidase

To determine the anatomical site of increased glucuronidase activity in kidney of K^+ -deficient mice, kidney sections were stained specifically for glucuronidase activity by using the substrate naphthol AS-BI glucuronide. Cells of the tubules of the medulla of 30-day- K^+ -deficient mice were greatly hypertrophied and the tubules dilated when compared with corresponding cells and tubules of normal mice (Plates 1a and 1b). Significant glucuronidase activity was present in the medulla but not the cortex of K^+ -deficient mice, and under the same staining conditions little was present in either the medulla or cortex of K^+ -replenished mice. Higher-power magnification of a medulla from deficient mice (Plate 1c) showed that much of the increased glucuronidase activity was in interstitial cells rather than in the epithelial cells of the collecting tubules themselves. Glucuronidase activity was especially prominent in interstitial cells in regions of the medulla bordering the cortex and was only rarely seen in the interstitial cells of the papillae. There was also an increase in glucuronidase activity in medullary-tubule cells of K^+ -deficient mice. This increased activity in the tubule cells is not readily apparent in Plates 1(b) and 1(c), because it is of a diffuse nature rather than being confined to discrete granules. Similar localizations of activity were

Fig. 3. *SDS/polyacrylamide-gel-electrophoresis patterns of labelled glucuronidase immunoprecipitated from kidney extracts of K⁺-deficient and K⁺-replenished mice*

Glucuronidase was immunoprecipitated from combined kidney extracts of three K⁺-deficient (○) or three K⁺-replenished (●) mice which had been labelled for 1 h with 300 μ Ci of [³H]leucine/mouse. The immunoprecipitates were electrophoresed in SDS/polyacrylamide gels and 1.25 mm slices were counted for radioactivity. The migration position of the authentic 73 000-mol. wt. glucuronidase subunit (73 000) and the Bromophenol Blue marker dye (BPB) are indicated. Molecular weight was estimated by using standard proteins of known molecular weight.

Table 3. *Rates of glucuronidase and total protein synthesis in kidney of K⁺-replenished (+) and K⁺-deficient (-) C57BL/6J mice*

Radioactivity incorporated into the 73 000-mol. wt. glucuronidase subunit was determined after SDS/polyacrylamide-gel electrophoresis of the immunoprecipitate from the combined kidney extracts of three mice as described in Fig. 3. Each value is the mean $\pm$ S.E.M. for the numbers of groups of mice indicated in parentheses. Values in the 'Day' column refer to the number of days for which mice were on K⁺-replenished or -deficient diet.

Day	Dietary K ⁺	Glucuronidase activity (units/g of kidney)	Radioactivity in glucuronidase (d.p.m./g of kidney) (a)	10 ⁻⁶ $\times$ Radioactivity in total cell protein (d.p.m./g of kidney) (b)	Relative rate of glucuronidase synthesis (10 ⁶ $\times$ a/b)
15	+	8.6	208 $\pm$ 13	27.8 $\pm$ 1.7	7.53 $\pm$ 0.48 (4)
	-	13.9	338 $\pm$ 35	30.0 $\pm$ 1.4	11.1 $\pm$ 0.99 (4)
22	+	8.1	173 $\pm$ 4.2	23.2 $\pm$ 1.1	7.45 $\pm$ 0.47 (5)
	-	16.2	411 $\pm$ 42	28.3 $\pm$ 1.7	14.5 $\pm$ 1.6 (4)
30	-	22.8	605 $\pm$ 82	54.8 $\pm$ 6.7	11.0 $\pm$ 0.53 (4)

apparent in kidney of 20-day-K⁺-deficient mice, although the staining was decreased in intensity and the number of foci of activity in interstitial cells was less. Also, similar glucuronidase localization was observed in kidneys of K⁺-deficient A/J and C57BL/6J beige mice, although individual lysosomes were larger in interstitial cells of beige mice (about 3–4 μ m and occasionally up to 10 μ m, compared with 1–1.5 μ m diameter in normal mice).

Discussion

These studies demonstrate that in the mouse K⁺ deprivation causes a 2–2.5-fold increase in kidney glucuronidase activity of all three inbred strains tested. Kidney glucuronidase increased linearly with time of K⁺ deprivation, at least up to 40 days. It is not possible to maintain the K⁺-deficient state much beyond this time, since loss of motor control and death

EXPLANATION OF PLATE I

Cellular localization of glucuronidase activity in kidney of K⁺-replenished and K⁺-deficient mice

Sections (8 μ m thick) were stained for 2h for glucuronidase activity with naphthol AS-BI β -D-glucuronide. (a) Kidney from C57BL/6J mice maintained on a K⁺-replenished diet for 30 days. Very few densely staining areas of glucuronidase activity are apparent in either the medulla (m) or the cortex (c). Bar = 100 μ m. (b) Kidney from C57BL/6J K⁺-deficient mice maintained on a K⁺-deficient diet for 30 days. Tubule cells of the medulla (m) are greatly distended in comparison with tubules of replenished mice. Densely staining areas of glucuronidase activity (arrows) are apparent throughout the interstitial cells of the medulla, but little activity is apparent in cells of the cortex (c). Bar = 100 μ m. (c) A higher-power magnification of medulla of K⁺-deficient C57BL/6J mice, illustrating glucuronidase activity (arrows) in interstitial cells of the medulla. Bar = 25 μ m.

rapidly follow. The increase in kidney glucuronidase specific activity probably would approach the 6-fold increase reported in acid phosphatase activity in papillae of kidneys of K^+ -deficient rats (Morrison & Panner, 1964) if activity was measured on dissected papillae of mice.

Among other organs tested there was a 2–3-fold increase in glucuronidase activity in heart of K^+ -deficient mice of the two C57BL/6J strains. The increase in heart glucuronidase activity, which occurred only in K^+ -deficient mice of the two C57BL/6J strains, is noteworthy in that Liebow *et al.* (1941) have reported a striking necrosis of myocardial tissue of the K^+ -deficient mouse. It is not known whether this increased activity is concentrated in heart cells or whether it is due to infiltrating leucocytes or fibroblasts, which even under normal conditions may form a significant portion of total heart lysosomal enzyme activity (Wildenthal *et al.*, 1975). Preliminary experiments suggest that the elevated heart glucuronidase activity in mice of the C57BL/6J strains is a recessive trait in that K^+ -deficient hybrid mice from a mating of strains C57BL/6J and A/J have normal heart glucuronidase activities (C. E. Cleveland & R. T. Swank, unpublished work). A possible reason for the unchanged lysosomal enzyme activity in other organs of K^+ -deficient mice is that some organs, such as liver, maintain normal cellular K^+ concentrations in spite of a severe fall in serum K^+ concentration (Gustafson *et al.*, 1973).

Although glucuronidase activity increased 2–2.5-fold in kidneys of K^+ -deficient mice, another lysosomal enzyme, β -galactosidase, did not significantly increase, and a third, acid phosphatase, increased by only 30%. Again it is possible that substantial increases in the enzymes occur in specific anatomical regions of the kidney. It is, of course, possible that deprivation of K^+ does increase the synthesis of these enzymes, but rapid degradation or secretion, as occurs with several kidney lysosomal enzymes in the androgen-treated mouse (Brandt *et al.*, 1975), prevents increases in kidney activity.

The present studies establish that the increase in kidney glucuronidase activity in kidney of K^+ -deficient mice is not regulated by either the *Gur* or *bg* (beige) genetic loci. Strain C57BL/6J is homozygous for the *Gur^b* allele, which lowers the androgen-mediated rate of synthesis of glucuronidase in kidney proximal tubules to one-third that found in strains such as A/J that are homozygous for the *Gur^a* allele. There is at most a 20% increase in kidney glucuronidase activity in K^+ -deficient C57BL/6J mice compared with A/J mice, and the slight increase is in the opposite direction to that expected for a *Gur^b* allele. Similarly there is only a slight (30%) increase in kidney glucuronidase in K^+ -deficient strain C57BL/6J beige mice compared with C57BL/6J mice. This

difference, however, is also seen in mice of the two strains maintained on K^+ -replenished diets, and is due to the lowered secretion of lysosomal enzymes from kidney proximal-tubule cells of beige mice (Brandt *et al.*, 1975; Brandt & Swank, 1976).

The antibody-titration experiments demonstrated that the mechanism for increased glucuronidase activity in kidney medulla of K^+ -deficient mice was accumulation of glucuronidase molecules. These experiments further showed that this accumulation was the result of an increased rate of synthesis of the enzyme. The absolute rate of glucuronidase synthesis increased in parallel with increased glucuronidase activity from day 15 to day 22 of K^+ depletion. The relative rate of glucuronidase synthesis after 30 days of K^+ depletion did not increase in parallel with enzyme activity. However, the 30-day experiments are difficult to interpret, because at this time some mice are starting to show pathological symptoms and a loss in body weight. We cannot, of course, exclude the possibility that small amounts of subunits of non-glucuronidase origin are in the 73000-mol.wt. peak, since present technology does not enable further characterization of these relatively small amounts of radioactivity. For such a postulated contaminant to account for the difference in rate of glucuronidase synthesis between normal and deficient mice, the degree of contamination would have to increase in K^+ -deficient mice. We consider it much more likely that the increased incorporation of radioactivity into the 73000-mol.wt. subunit in K^+ -deficient mice is due to increased glucuronidase synthesis, which, in turn, causes parallel accumulation of catalytically active enzyme.

Another possible reason for the increased enzyme activity in kidney of K^+ -deficient mice might be lowered secretion from kidney into urine. We found that equally low amounts of enzyme (2–4% of total kidney content) were daily secreted from both K^+ -replenished and K^+ -deficient mice (C. E. Cleveland & R. T. Swank, unpublished work). However, it is unknown whether this small amount of enzyme originates in the kidney or in other tissues of the urogenital tract.

The cellular sites for increased glucuronidase activity in K^+ -deficient mice are similar to the sites of accumulation of lysosomes in K^+ -deficient rats. That is, there is increased activity in the collecting tubules of the entire medulla, including the papilla and cells nearer the border with the cortex. However, in 20-day and 30-day K^+ -deficient mice a large amount of activity is present in interstitial cells, in all regions of the medulla except for the papilla. It is likely therefore that a significant portion of the increased lysosomal activity and increased rates of synthesis, particularly after long periods of K^+ depletion, is due to infiltration of cells, such as macrophages, polymorphonuclear leucocytes or fibroblasts, which synthesize

lysosomal enzymes at a higher rate than do resident kidney cells. Phagocytic cells of these types would be expected to respond to the slightly necrotic state induced in kidney by long-term K^+ depletion (Liebow *et al.*, 1941).

In summary, the K^+ -deficient mouse responds similarly to the K^+ -deficient rat in that the activities of some kidney acid hydrolases are significantly elevated and similar cell types of the medulla are affected. Whether increased synthesis of lysosomal enzymes is also the mechanism for increased lysosomal enzyme activity in kidney of K^+ -deficient rats is unknown. With regard to the utility of the K^+ -deficient mouse kidney system for the study of lysosomal biogenesis, it is clear that the lysosomal amplification is not as dramatic as, for example, the high concentration of glucuronidase found in the rat preputial gland (Ohtsuka & Wakabayashi, 1970; Keller & Touster, 1975) or the androgen-mediated 20–50-fold increase in activity of glucuronidase in mouse kidney (Fishman, 1965; Swank *et al.*, 1973), so that studies of turnover of lysosomal enzymes will be difficult. Also mutations that have proved useful in studies of lysosome function in other systems apparently have no effect in this system. A further caution, at least with regard to lysosomal enzyme activity of K^+ -deficient mice, is that infiltration of lysosome-rich interstitial cells appears to be partially responsible for the increased activity, especially at advanced stages of K^+ depletion.

We thank Dr. E. J. Brandt for helpful discussions and Mr. Gerald Jahreis for skilful and dedicated technical assistance. We are indebted to Dr. Roger Ganschow and Dr. Ross Davey, who perfected much of the technique for measurements by immunoprecipitation of rates of glucuronidase synthesis. The work was supported in part by U.S. Public Health Service Grant no. GM-19521.

References

- Aithal, H. N., Toback, F. G., Dube, S., Getz, G. S. & Spargo, B. H. (1977) *Lab. Invest.* **36**, 107–113
- Brandt, E. J. & Swank, R. T. (1976) *Am. J. Pathol.* **82**, 573–588
- Brandt, E. J., Elliott, R. W. & Swank, R. T. (1975) *J. Cell Biol.* **67**, 774–788
- Fishman, W. H. (1965) *Methods Horm. Res.* **4B**, 273–326
- Gustafson, A. B., Shear, L. & Gabuzda, G. J. (1973) *J. Lab. Clin. Med.* **82**, 287–296
- Hayashi, M. (1964) *J. Histochem. Cytochem.* **12**, 293–297
- Hers, H.-G., Berthet, J., Berthet, L. & de Duve, C. (1951) *Bull. Soc. Chim. Biol.* **33**, 21–41
- Keller, R. K. & Touster, O. (1975) *J. Biol. Chem.* **250**, 4765–4769
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Liebow, A. A., McFarland, W. J. & Tennant, R. (1941) *Yale J. Biol. Med.* **13**, 523–538
- Manitius, A. H., Beck, L. D. & Epstein, F. H. (1960) *J. Clin. Invest.* **39**, 684–692
- Morrison, A. B. & Panner, B. J. (1964) *Am. J. Pathol.* **45**, 295–311
- Muehrcke, R. C. & Rosen, S. (1964) *Lab. Invest.* **13**, 1359–1373
- Ohtsuka, K. & Wakabayashi, M. (1970) *Enzymologia* **39**, 109–124
- Oliver, C. & Essner, E. (1973) *J. Histochem.* **21**, 218–228
- Paigen, K., Swank, R. T., Tomino, S. & Ganschow, R. E. (1975) *J. Cell. Physiol.* **85**, 379–392
- Prieur, D. J., Young, D. M., Reagan, R. L. & Olson, H. M. (1973) *Proc. Soc. Exp. Biol. Med.* **144**, 683–687
- Spargo, B., Straus, F. & Fitch, M. D. (1960) *Arch. Pathol.* **70**, 599–613
- Swank, R. T., Paigen, K. & Ganschow, R. E. (1973) *J. Mol. Biol.* **81**, 225–243
- Toback, F. G., Ordenez, N. G., Bortz, S. L. & Spargo, B. H. (1976) *Lab. Invest.* **34**, 115–124
- Wildenthal, K., Kao, R., Rannels, D. E. & Morgan, M. (1975) in *Lysosomes in Biology and Pathology* (Dingle, J. T. & Dean, R. T., eds.), vol. 4, pp. 167–190, North-Holland, Amsterdam and Oxford
- Wilson, H., Spargo, M. D. & Getz, G. S. (1973) *Am. J. Pathol.* **71**, 315–330