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Lysosomal glycolipid storage in the renal tubular epithelium in mastomys (*Praomys coucha*)

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Summary. The renal proximal tubular epithelium of MCC strain of mastomys (*Praomys coucha*) exhibited a number of cytoplasmic vacuoles after conventional paraffin-embedding procedures. These vacuoles were strongly PAS-positive in cryostat sections. Ultrastructurally, they were double membrane-bound structures filled with myelin figures and acid phosphatase-positive electron-dense matrix. Immunofluorescent microscopy revealed that these structures contained GM_2 ganglioside. Other tissues or organs were histologically normal. Mating experiments indicated that the ganglioside storage in MCC mastomys is inherited as an autosomal recessive trait.

Key words: Kidney, Lysosome, Mastomys, Storage disease

Introduction

Mastomys (*Praomys coucha*) is an African rodent which is intermediate in size between the mouse and rat. Since the first introduction of this species into laboratories in the 1940s, it has been used in many biomedical fields, including oncology, parasitology, and epidemiology (Solleveld, 1987). One of the advantages of using mastomys as a laboratory rodent is that it exhibits great phenotypic variations, as do the laboratory mouse and rat. An inbred strain of mastomys, MCC, with pink eyes and diluted hair colour, was reported to have the green-brown kidney (Tanaka et al., 1988). We found that the renal proximal tubule epithelial cells of this strain have many cytoplasmic vacuoles after conventional paraffin-embedding procedures. Here we report these vacuoles to be large lysosomes containing glycolipids (gangliosides), as determined by histochemical, immunohistochemical and electron microscopic techniques.

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Materials and methods

Animals

Animals were raised under conventional conditions and provided with water and commercial laboratory mouse chow ad libitum. Ten MCC mastomys (5 males and 5 females), aged 23-244 days, were used in this study. Wild-coloured mastomys (MST) with normal kidneys (Ogura et al., 1992) were used as control at nearly the same age as MCC.

Light microscopy

Formalin-fixed kidneys were routinely processed and embedded in paraffin. Sections (4 μm thick) were stained with haematoxylin-eosin (H&E). Frozen sections from formalin-fixed specimens were stained with periodic acid Schiff (PAS) or Oil red O.

Electron microscopy

Small pieces (about 0.5 mm thick) of renal cortex were fixed with 2% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 2 h and postfixed with 1% osmium tetroxide for 2 hrs. After dehydration, the specimens were embedded in a mixture of Araldite and epoxy-resin. Ultrathin sections (60 nm thick) were stained with uranyl acetate and lead citrate, and examined with a Hitachi H-7000 electron microscope. For the detection of acid phosphatase at the electron microscopic level, the specimens were processed according to Robinson and Karnovsky (1983). Briefly, after fixation with 2% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) containing 5% sucrose for 30 min, the specimens were sliced at a thickness of 60 μm with a microslicer. The slices were incubated with an acid phosphatase-reaction mixture for 90 min at 37 ºC with gentle shaking. After washing with cacodylate buffer, the slices were fixed and processed for electron microscopy as described above, except that sections were stained with uranyl acetate only.

Immunohistochemistry

Our preliminary biochemical analysis demonstrated a high content of a ganglioside (GM_2) in the homogenate of the kidney of MCC mastomys as compared with that of MST mastomys. To determine whether the vacuoles in the kidney of MCC mastomys are the sites of the GM_2 accumulation, we performed fluorescent immunohistochemistry with an avian antibody to GM_2 ganglioside. Frozen sections (5 µm thick) were cut from fresh renal cortex and fixed with acetone for 30 seconds. They were incubated with an avian antibody to GM_2 ganglioside (Kasai et al., 1985) diluted to 1:10 in PBS for 18 h at 4 °C, followed by incubation with an FITC-conjugated rabbit anti-chicken IgG (E.Y. Lab., San Mateo, CA) diluted to 1:50 in PBS for 3 h at 25 °C.

Mating experiments

Mating experiments were performed between MCC strain and MST strain (normal) of mastomys to determine the mode of inheritance of vacuolation of the renal tubules in MCC mastomys.

Results

In paraffin-embedded sections stained with H&E, many round vacuoles of varying sizes were found in the proximal tubule epithelial cells (Fig. 1). The vacuoles increased in size and number with age, and filled the entire cytoplasm by 80 days of age in both sexes. In frozen sections, the contents of the vacuoles were strongly positive when stained with PAS (Fig. 2) and were negative when stained with Oil red O (results not shown). Ultrastructurally, the cytoplasm of the proximal tubule epithelial cells was filled with many round, double membrane-bound structures which contained multilamellar myelin figures and electron-dense amorphous matrix (Fig. 3). The myelin figures in the myelinoid bodies were arranged as unicentric and multicentric whorls, or as stacks (Fig. 4). These structures resembled the «myelinoid bodies» (or myelinosomes) reported previously (Ghadially, 1982). Considering their size and number within the proximal tubule epithelial cells, the myelinoid bodies probably correspond to the vacuoles observed in paraffin-embedded sections. The older the animals were, the greater the volume and number of the myelinoid bodies. Neither the renal distal tubules nor the glomeruli had notable structural abnormalities.

To confirm whether the large myelinoid bodies in MCC mastomys were lysosomes, the proximal tubule epithelial cells were examined for acid phosphatase at the ultrastructural level. The myelinoid bodies contained electron-dense reaction products within their matrix (Fig. 5), indicating that they were lysosomes. In MST mastomys, normal-sized lysosomes were positive for acid phosphatase reaction (results not shown).

Immunohistochemistry with an antibody against GM_2 showed positive staining within the cytoplasm of the proximal tubule epithelial cells (Fig. 6).

The results of mating experiments are presented in Table 1. Two pairs of female MST mastomys and male MCC mastomys produced 20 offspring, all of which had normal kidneys. Matings of the F1 mastomys produced 84 offspring; 61 had normal kidneys and 23 had affected kidneys. This segregation ratio did not deviate significantly from the 3:1 ratio expected on the basis of a single recessive mutation. No significant interaction with sex was indicated.

Table 1. Result of matings of MST and MCC mastomys.

MATING PARENTS	OBSERVED		EXPECTED			
	Normal	Affected	Normal	Affected	χ^2	p
MST x MCC						
Male	10	0	10	0		
Female	10	0	10	0		
Total	20	0	20	0		
F1 x F1						
Male	38	16	40.5	13.5	0.1	0.75
Female	23	7	22.5	7.5	0.02	0.88
Total	61	23	63	21	0.03	0.86

Discussion

In general, the observation of cytoplasmic vacuoles after a conventional paraffin-embedding procedure

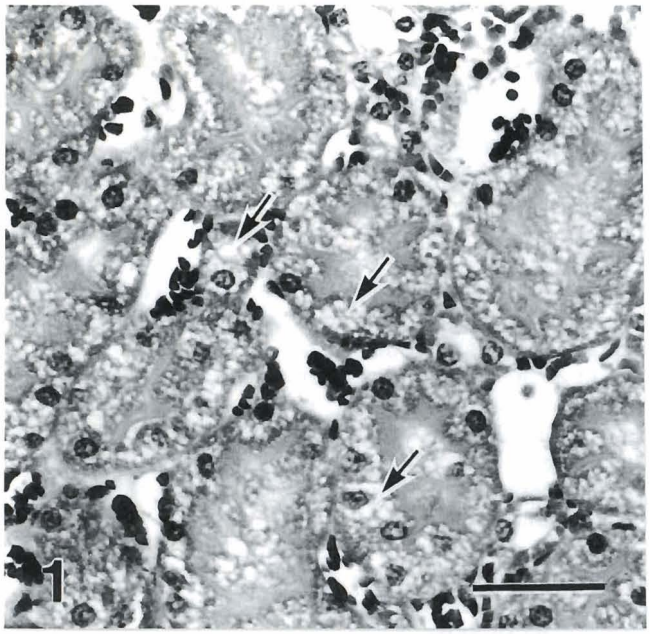
Fig. 1. Light photomicrograph of the renal cortex of a 150-day-old male mastomys. Haematoxylin-eosin staining. Numerous vacuoles (arrows) are seen in the proximal tubule epithelium. Bar: 50 µm.

Fig. 2. Light photomicrograph of the renal cortex of a 150-day-old male mastomys. PAS staining. The contents of vacuoles are well preserved in cryostat sections. They are PAS-positive (arrows). Bar: 50 µm.

Fig. 3. Electron photomicrograph of the renal proximal tubules of a 150-day-old male mastomys. A large number of myelinoid bodies are scattered throughout the epithelial cells (arrows). Bar: 3 µm.

Fig. 4. High-power view of Fig. 3. The myelin figures in myelinoid bodies are arranged as concentric whorls (arrow) or as stacks (long arrow). Bar: 1 µm.

Fig. 5. Electron photomicrograph of the renal proximal tubules of a 150-day-old male mastomys. Acid phosphatase reaction. The matrix of the myelinoid bodies is positively stained (arrows). Bar: 1 µm.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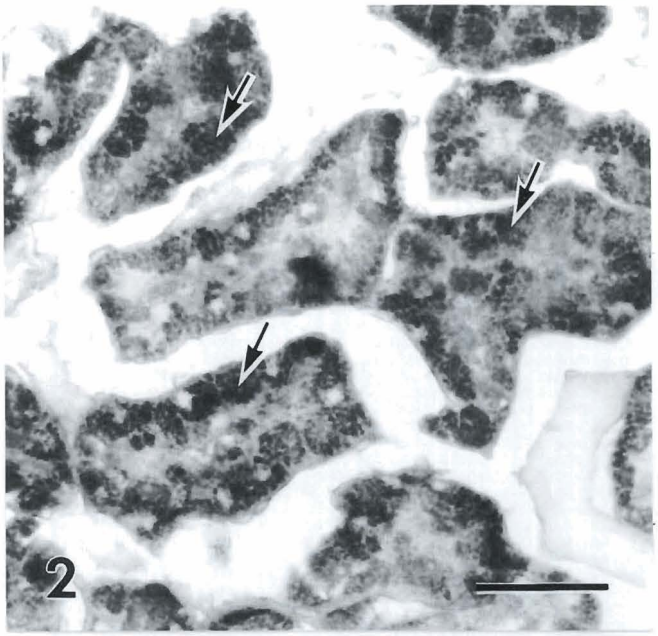
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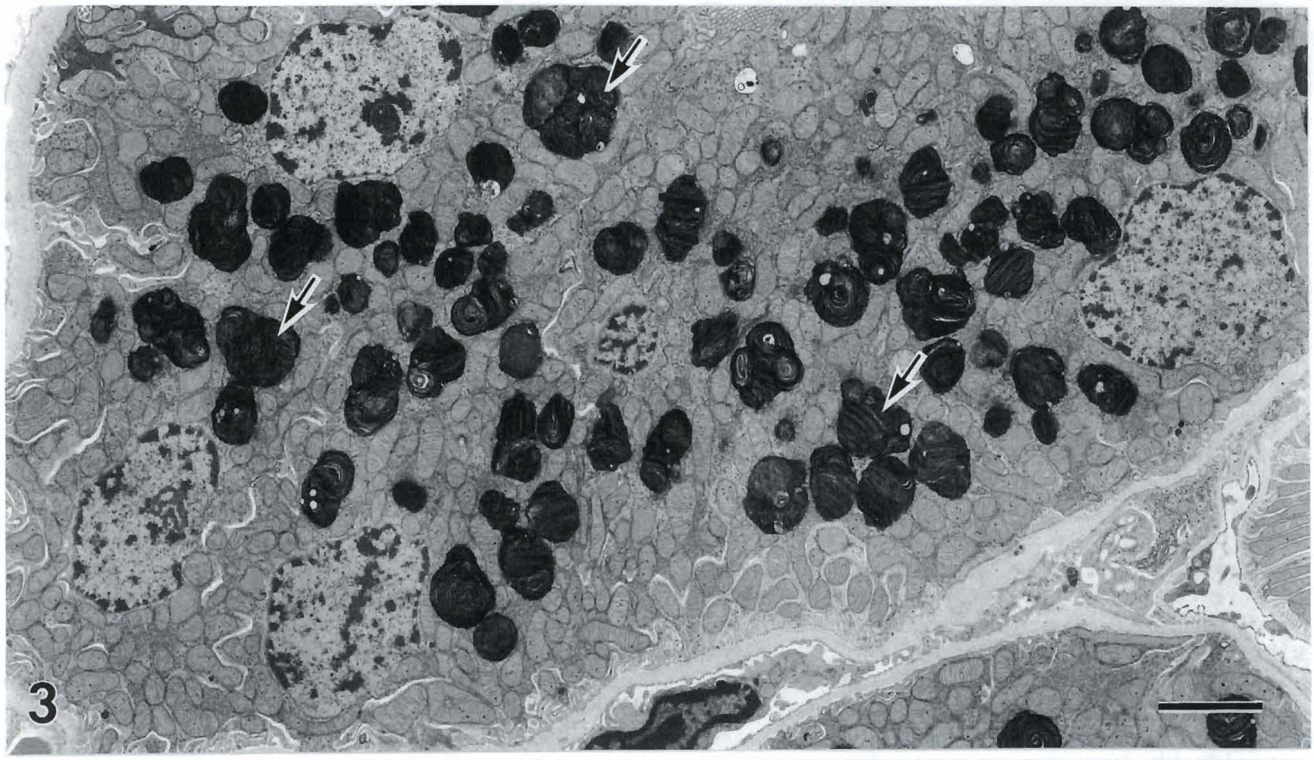
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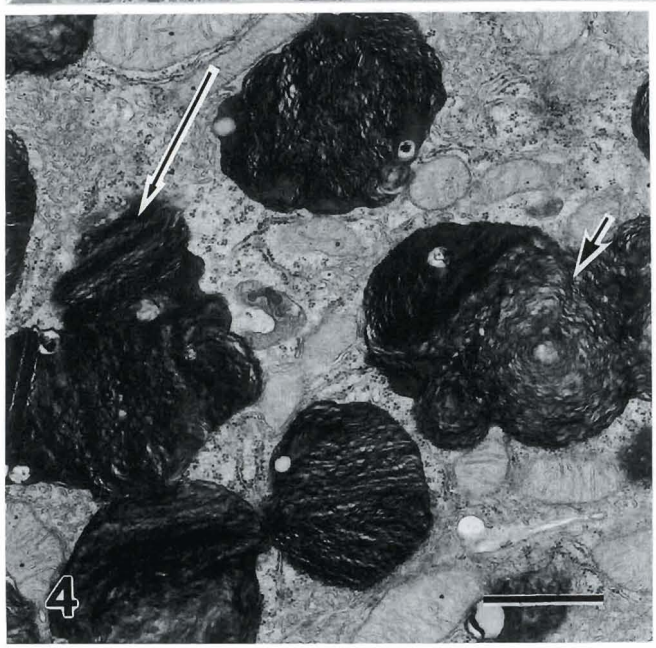
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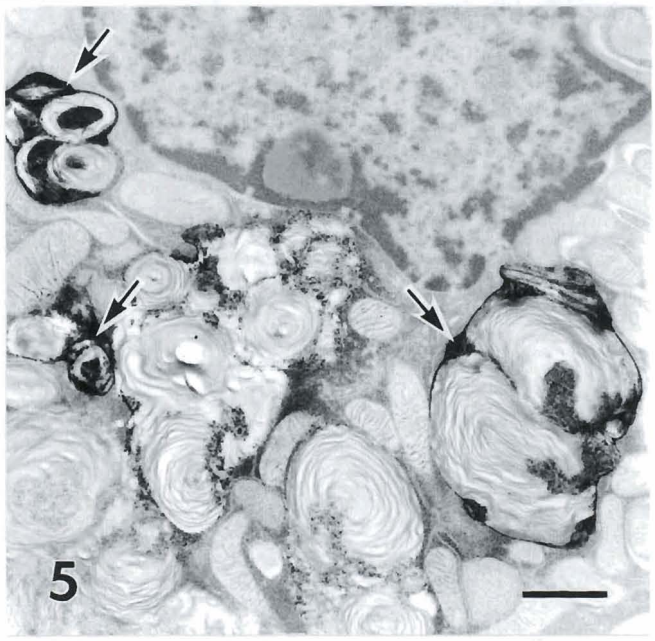
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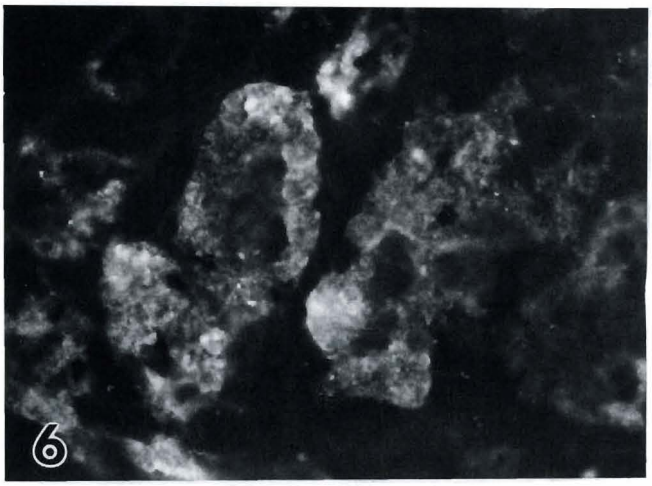

Fig. 6. Immunofluorescent photomicrograph of the renal cortex of a 100-day-old female mastomys. The cytoplasm of proximal tubule epithelial cells is positively stained with an antibody against a ganglioside (GM_2). Bar: 50 μm.

indicates an excessive accumulation of lipids in the cytoplasm. In the case of MCC mastomys, the vacuoles in the renal proximal tubule epithelium were not lipid droplets since they were negative for Oil red O. The ultrastructural examination combined with detection of acid phosphatase demonstrated that cytoplasmic vacuoles in the renal proximal tubular epithelium in MCC mastomys were myelinoid bodies, i.e., large lysosomes containing myelin figures. Myelinoid bodies have been reported to appear in some inherited lysosomal storage diseases, which occur as a result of a deficiency or absence of a certain lysosomal enzyme or a lysosomal transport disorder (Ianchu, 1992). In MCC mastomys, lysosomes of the renal proximal tubular epithelium accumulated PAS-positive lipids (glycolipids), which included, at least in part, GM_2 ganglioside. With regard to ganglioside storage (gangliosidosis), it is very common that the neural system is affected, and that neurological symptoms appear. The ganglioside storage in MCC mastomys is unique in that the pathological alteration was confined to the proximal tubular epithelium of the kidney and that animals showed no clinical signs.

The mating experiments indicated that the ganglioside storage in MCC mastomys was inherited as an autosomal recessive trait. It would be of interest in the future to clarify the mechanism by which this mutant gene affects metabolic pathways of gangliosides in the renal tubular epithelium.

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References

Ghadially F.N. (1982). Lysosomes. In: Ultrastructural pathology of the cell and matrix. 2nd ed. Butterworths Co. London. pp 435-579.

Ianchu T.C. (1992). The ultrastructural spectrum of lysosomal storage diseases. Ultrastruct. Pathol. 16, 231-244.

Kasai N., Naiki M., Ariga T., Hirabayashi Y. and Yu R.K. (1985). Preparation and specificity of avian anti-GM_2(NeuGc) ganglioside antiserum. Biochem. Biophys. Res. Commun. 129, 334-341.

Ogura A., Asano T., Noguchi A., Mochida K. and Suzuki O. (1992). Ultrastructure of lipid storage cells in the adrenal cortex and the ovary in female mastomys (*Praomys coucha*). Vet. Pathol. 29, 253-255.

Robinson J.M. and Karnovsky M.J. (1983). Ultrastructural localization of several phosphatases with cerium. J. Histochem. Cytochem. 31, 1197-1208.

Solleveld H.A. (1987). The multimammate mouse. In: The UFAW handbook on the care and management of laboratory animals. 6th ed. Pool T. (ed). Logman Scientific & Technical. Essex. pp 346-359.

Tanaka S., Nozaki M., Maruyamauchi T., Kaneko T., Yamanouchi K., Okugi M. and Matsuzawa A. (1988). Laboratory strains of hamster, gerbil and mastomys maintained at the Institute of Medical Science, University of Tokyo. Jpn. J. Exp. Med. 58, 273-278.

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