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Intracytoplasmic Granules of the Inner Medulla and Papilla of the Potassium Depleted Human Kidney

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Summary. Morphological changes were studied in the kidney of a potassium depleted 21-year old Japanese male with Bartter's syndrome. Characteristic features were PAS-positive intracytoplasmic granules in the inner medulla and the papilla. The intracytoplasmic granules were conspicuous towards the papillary tip and ultrastructurally composed of small vesicles, vacuoles, amorphous dense materials, lamellar contents and myelin like figures, and bounded by a single limiting membrane. The granules in this human kidney were, with regard to their distribution and ultrastructural findings, similar to those in the kidney of experimentally potassium depleted rats. The results indicate that the intracytoplasmic granules in the renal inner medulla and the papilla are the characteristic feature of the potassium depleted human kidney and a counterpart to those in the potassium depleted rat.

Key words: Hypokalaemia – Bartter's syndrome – Kidney medulla – Intracytoplasmic granules

Introduction

Potassium depletion produces morphological changes in the kidneys of man and animals. In the experimentally potassium depleted rat, characteristic lesions are an accumulation of intracytoplasmic granules in the renal papilla and the inner medulla (Oliver et al. 1957; Spargo et al. 1960; Morrison et al. 1963; Panner and Morrison 1963; Muehrcke and Rosen 1964), and an adenomatous hyperplasia of the epithelium of the collecting tubules in the inner stripe of the outer medulla (Oliver et al. 1957; Wachstein and Meisel 1959; Toback et al. 1976). The renal histological change in potassium depleted humans is characterized by a vacuolar change of the renal tubules, especially of the proximal convoluted tubules, in the renal cortex (Relman and Schwartz 1956; Muehrcke and Rosen 1964). It has been considered that the intracytoplasmic

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granules seen in the renal inner medulla and the papilla of the rat with potassium depletion are absent in potassium depleted humans (Heptinstall 1974). France et al. (1962, 1973, 1974, 1978), however, called attention to the appearance of intracytoplasmic granules in the renomedullary cells of the potassium depleted patients, but ultrastructure of these intracytoplasmic granules has not been examined properly. The purpose of this paper is to report and compare light and electron microscopic findings in the kidney of a potassium depleted patient with Bartter's syndrome, with those in the experimentally potassium depleted rat.

Materials and Methods

A 21-year old Japanese man was admitted to Kyushu University Hospital with a history of intermittent weakness of the extremities for six months. He was diagnosed of Bartter's syndrome on the basis of the following findings; muscular weakness, normal blood pressure (B.P. 104/56) hypokalaemia (1.9 mEq/L), elevated plasma renin activity (15.4 ng/ml/h), elevated plasma aldosterone concentration (50 ng/dl), no pressure response to angiotensin II, metabolic alkalosis (pH 7.45, HCO_3^- 27.6 mEq/L) and hyperplasia of the juxtaglomerular apparatus. Treatment with indomethacin resulted in an increase of serum potassium concentration from 2.0 to 3.8 mEq/L with decreasing urinary potassium excretion.

Light and electron microscopic observations were performed on renal biopsies, which were taken before and after treatment with indomethacin. Paraffin sections were stained with haematoxylin-eosin, periodic acid-Schiff (PAS) and PAS-alucian blue. For electron microscopy, the samples were embedded in epon. Semithin sections were stained with toluidine blue and ultrathin sections were contrasted by uranyl acetate and lead citrate.

For comparison with the human kidney, 8 Wistar male rats weighing between 160 and 200 g were depleted of potassium by feeding them a virtually potassium-free diet. One, 2 and 4 weeks after starting potassium depletion, 2, 4 and 2 rats were killed respectively, and their kidneys were examined. Two other rats given free access to normal diet were used as controls. Kidneys were fixed by an arterial perfusion method (Griffith et al. 1967; Schwartz and Venkatachalam 1974). For light microscopy paraffin sections were stained with haematoxylin-eosin and PAS. For electron microscopy the tissues were embedded in epon. Semithin sections were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a JEM 100CX electron microscope.

Results

1. Morphological Findings in the Potassium Depleted Human Kidney

By light microscopy, widening of juxtaglomerular areas and an increase in the number of juxtaglomerular cells were recognized in the cortex. Vacuolar change of the proximal convoluted tubules in the cortex was not a feature. In the renal medulla intracytoplasmic granules, which were eosinophilic and positive with PAS and toluidine blue, were conspicuous (Fig. 1). These granules, which varied in size and intensity of staining, were found in the epithelium of the collecting tubules, the vascular endothelium, the interstitial cells and the epithelium of Henle's loops. The accumulation of intracytoplasmic granules was more prominent in the first three, increasing in intensity towards the papillary tip. These granules were more easily recognized on the side of luminal surface of the cytoplasm. In addition, the collecting tubules showed proliferation

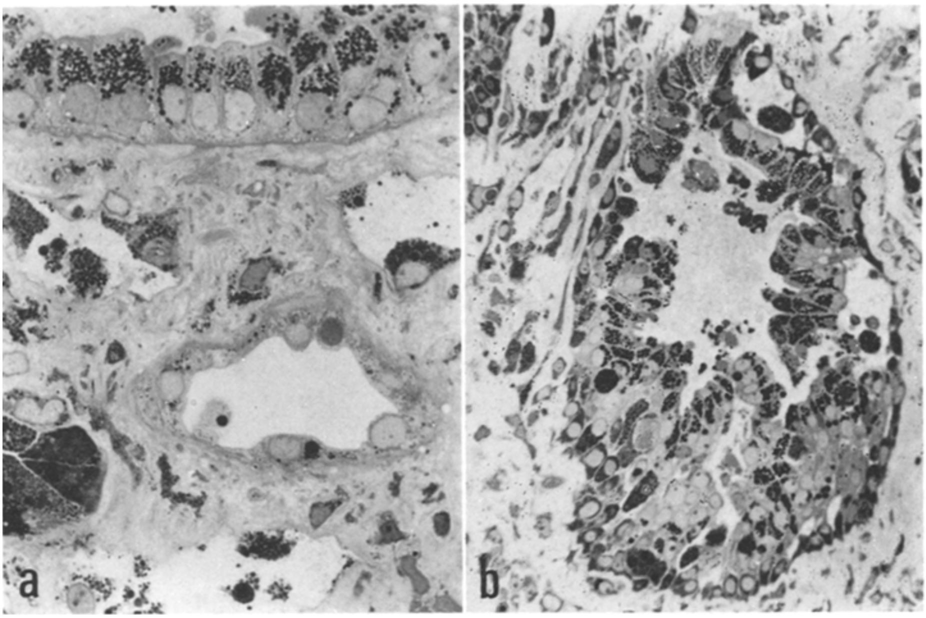

Fig. 1 a, b. Histological findings of the renal papilla of potassium depleted patient. **a** Intracytoplasmic granules in the epithelium of collecting tubule (*top*), Henle's loop (*center*), vascular endothelium (*left*) and interstitial cells. (Toluidine blue ×600) **b** Collecting tubule located near the papilla. Epithelial cells are hyperplastic and filled with numerous granules (Toluidine blue ×290)

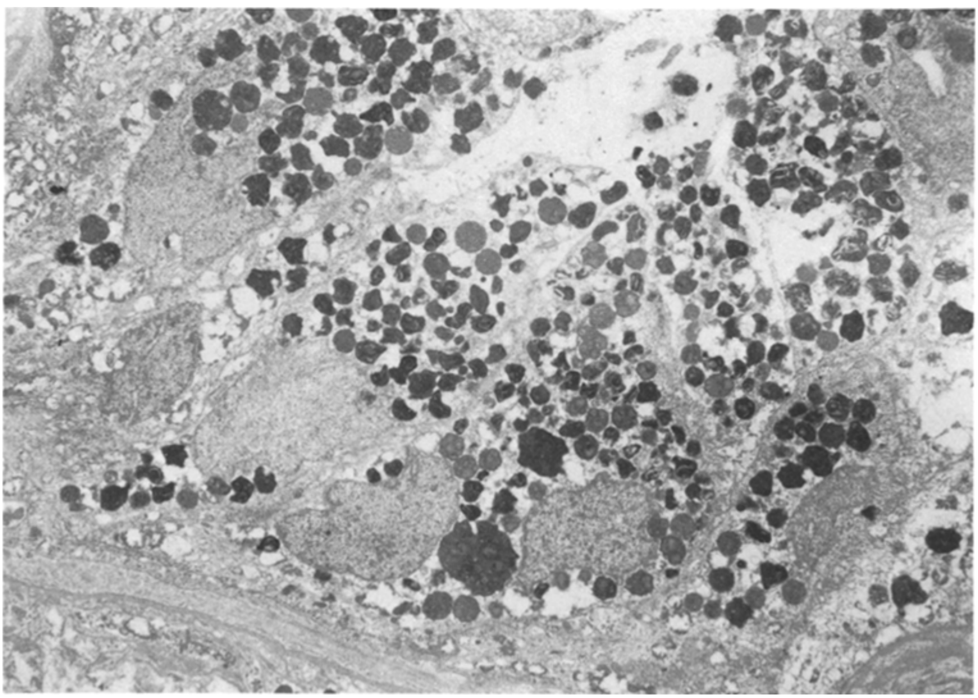

Fig. 2. Electron micrograph showing collecting tubule in the papilla of potassium depleted patient. Note intracytoplasmic granules accumulating in the luminal side of the cytoplasm (×3,200)

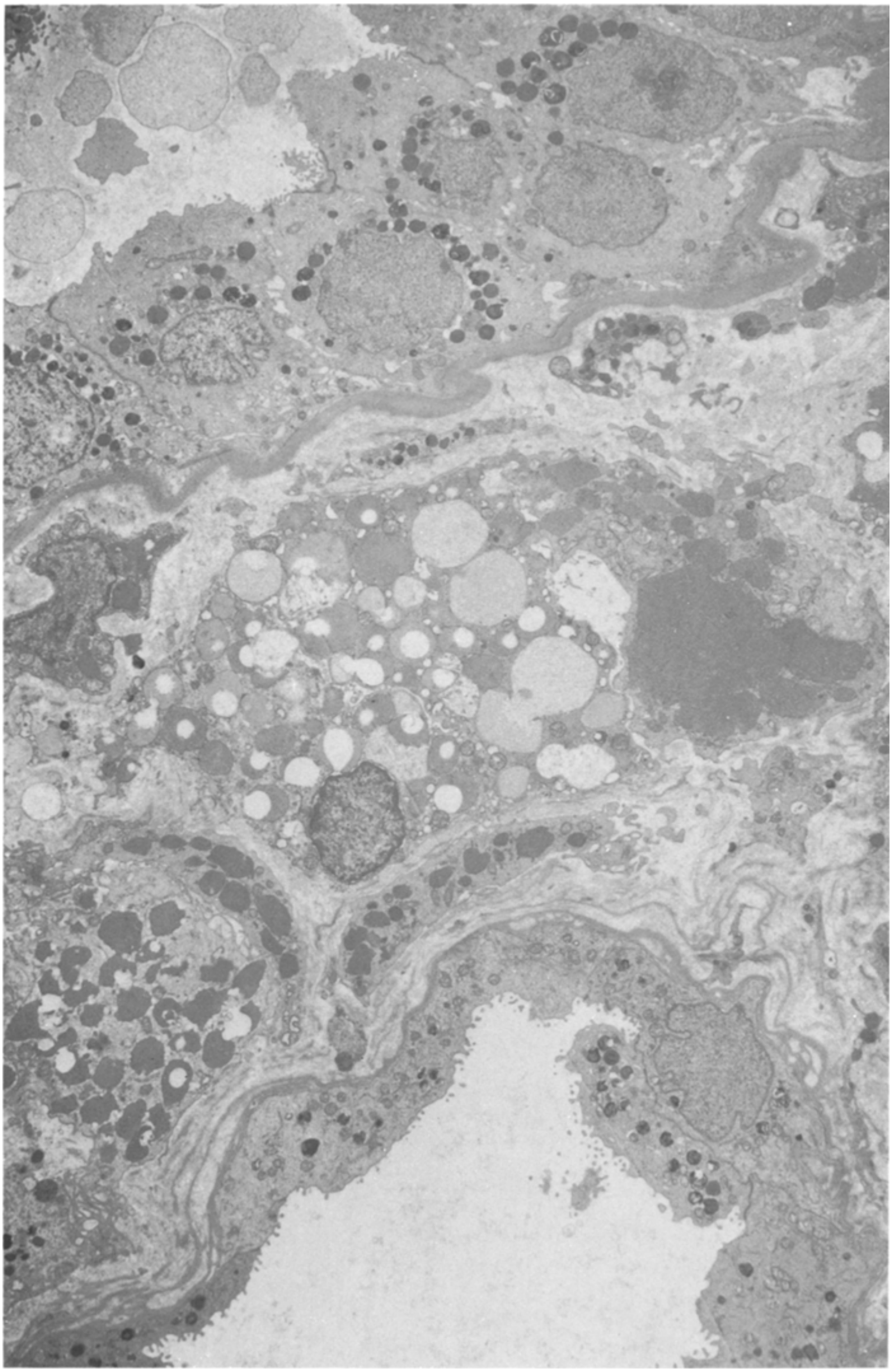

Fig. 3. Intracytoplasmic granules in collecting tubule (*top*), interstitial cells (*center*) and Henle's loop (*bottom*) of renal medulla of potassium depleted patient. Granules in the interstitial cells are more amorphous and contain large vacuoles (×3,250)

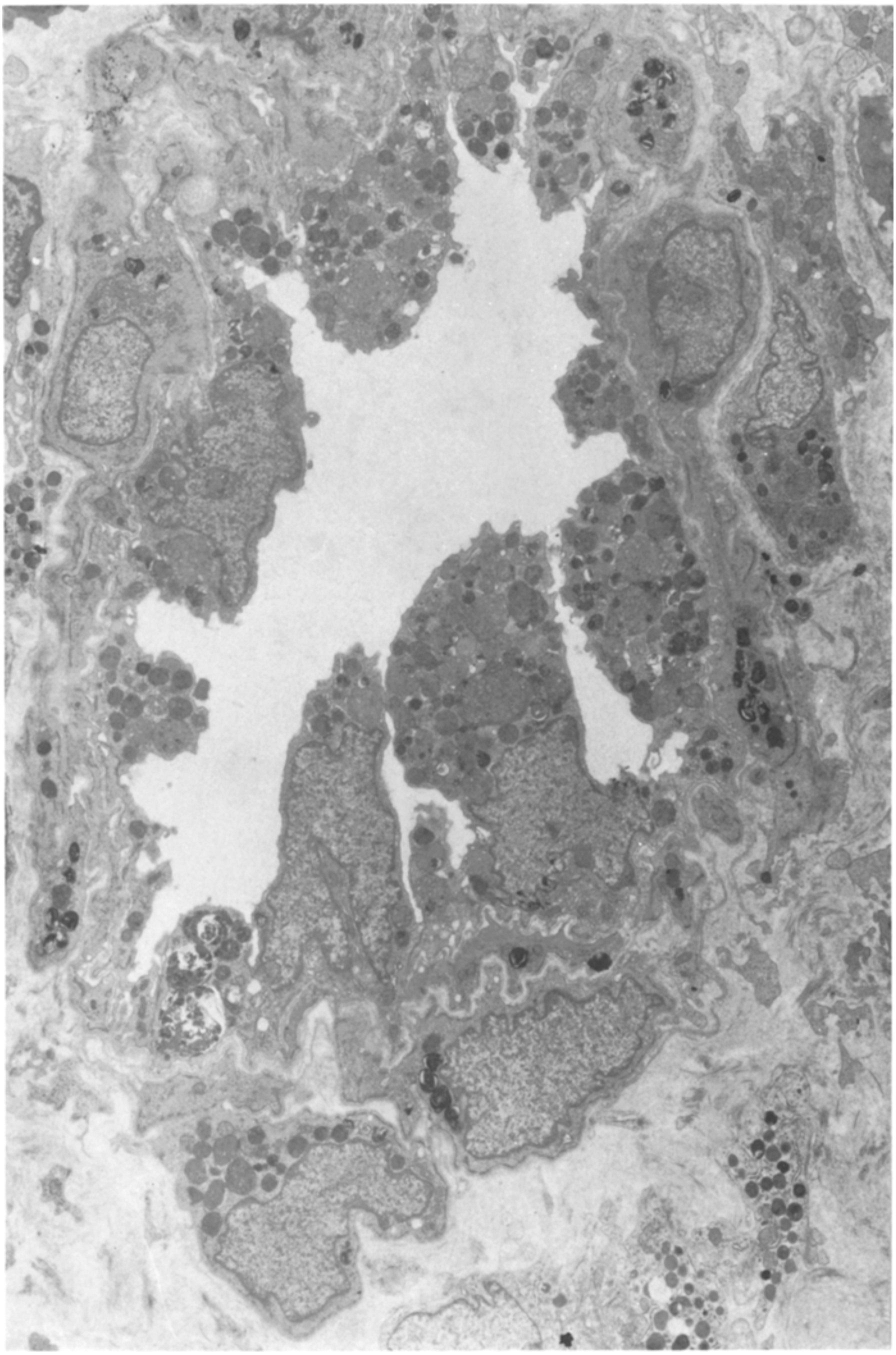

Fig. 4. Small artery of renal medulla of potassium depleted patient. Intracytoplasmic granules present in endothelial cells and smooth muscle cells. Endothelial cells contain numerous granules and their swollen cytoplasm protrudes into the lumen. Note also hypergranulation of interstitial cell cytoplasm (*bottom right*) (× 4,000)

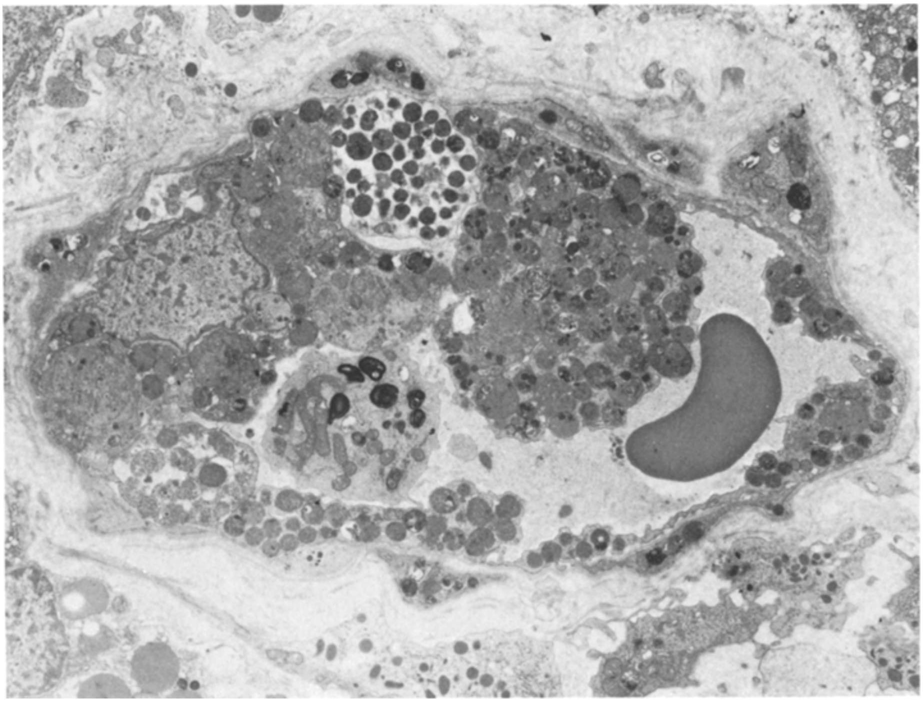

Fig. 5. Small blood vessel in the renal medulla of potassium depleted patient. Endothelial cells filled with granules are swollen and markedly narrow the lumen. Smooth muscle cells contain some granules (×3,700)

of epithelial cells (Fig. 1b) and there was an increase in the number of interstitial cells in the medulla. After treatment with indomethacin raised the serum potassium level the accumulation of the PAS positive intracytoplasmic granules was notably decreased.

Electron microscopic study of the renal medulla revealed a striking accumulation of osmiophilic granules in all cell types (Figs. 2, 3, 4 and 5). These intracytoplasmic granules were bounded by a single limiting membrane and varied in size, osmiophilia and internal structure. The components included were numerous vesicles, vacuoles, lamellar contents, dense clumps of amorphous material and myelin like figures (Fig. 6). Neither intermediate forms between these granules and mitochondria were seen nor were cytoplasmic organelles related to the intracytoplasmic osmiophilic granules. In interstitial cells, not only these intracytoplasmic granules but also the more dense amorphous droplets, which were identical with those of lipid droplets present in interstitial cells of the normal kidney, were markedly increased (Figs. 3, 6c, d). The epithelium of the collecting tubules was hyperplastic and filled with numerous granules, preferentially accumulated in the luminal side of the cytoplasm (Fig. 2). Vascular endothelial cells were also swollen and protruded into the lumen, seemingly owing to the granular change of the cytoplasm. Similar granules were also noted in the cytoplasm of smooth muscle cells (Figs. 4 and 5).

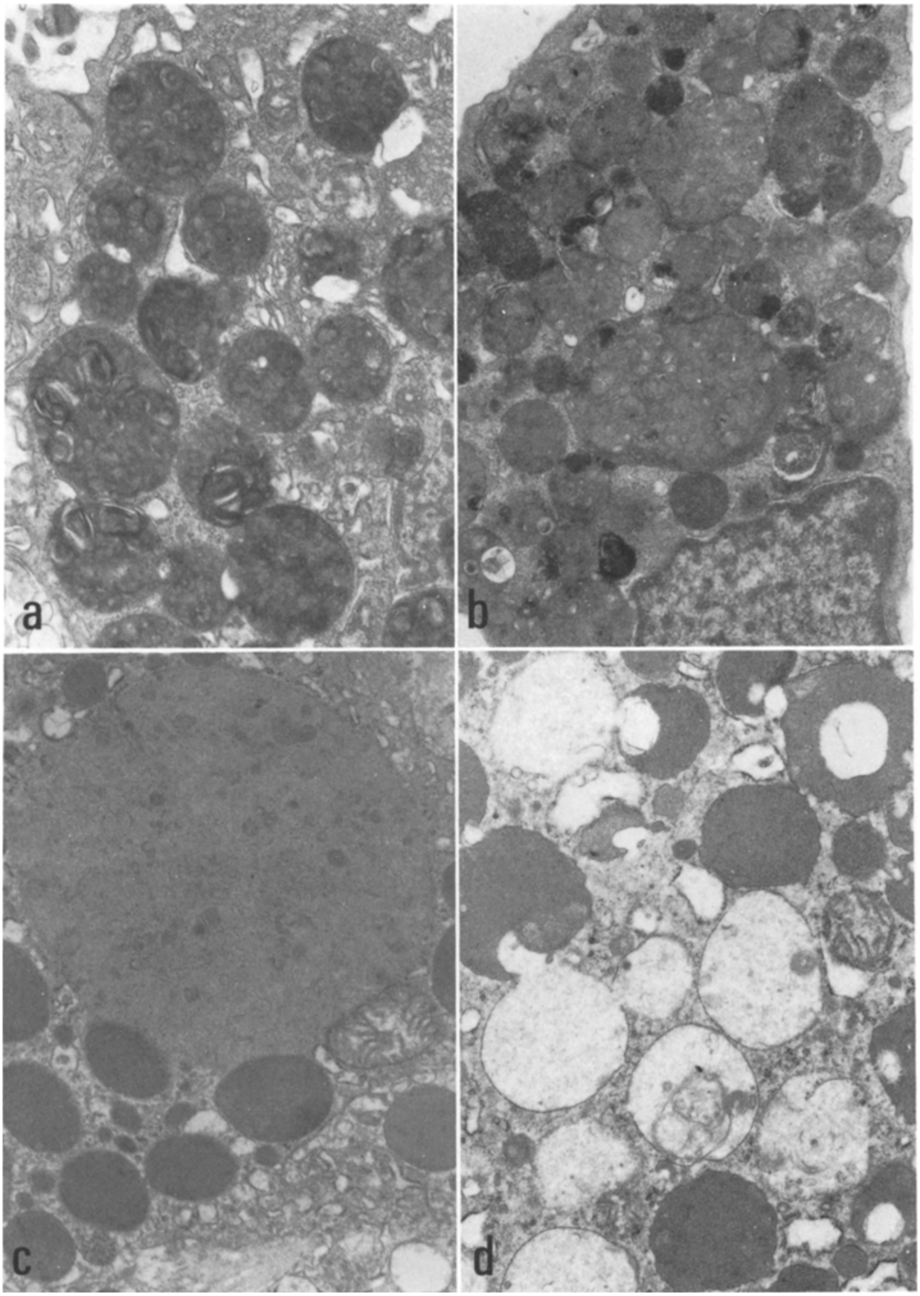

Fig. 6a–d. Intracytoplasmic granules of renomedullary cells of potassium depleted patient. **a** Granules of collecting tubule containing myelin like figures are bounded by a single limiting membrane (×13,200). **b** Granules of vascular endothelium are composed of vesicles, vacuoles and lamellar contents (×12,000). **c** Granules of interstitial cell. A large granule contains lamellar structures and others are packed with dense amorphous materials (×10,560). **d** Most granules of interstitial cell appear densely amorphous but contain variable number of lamellar contents or vacuoles (×12,000)

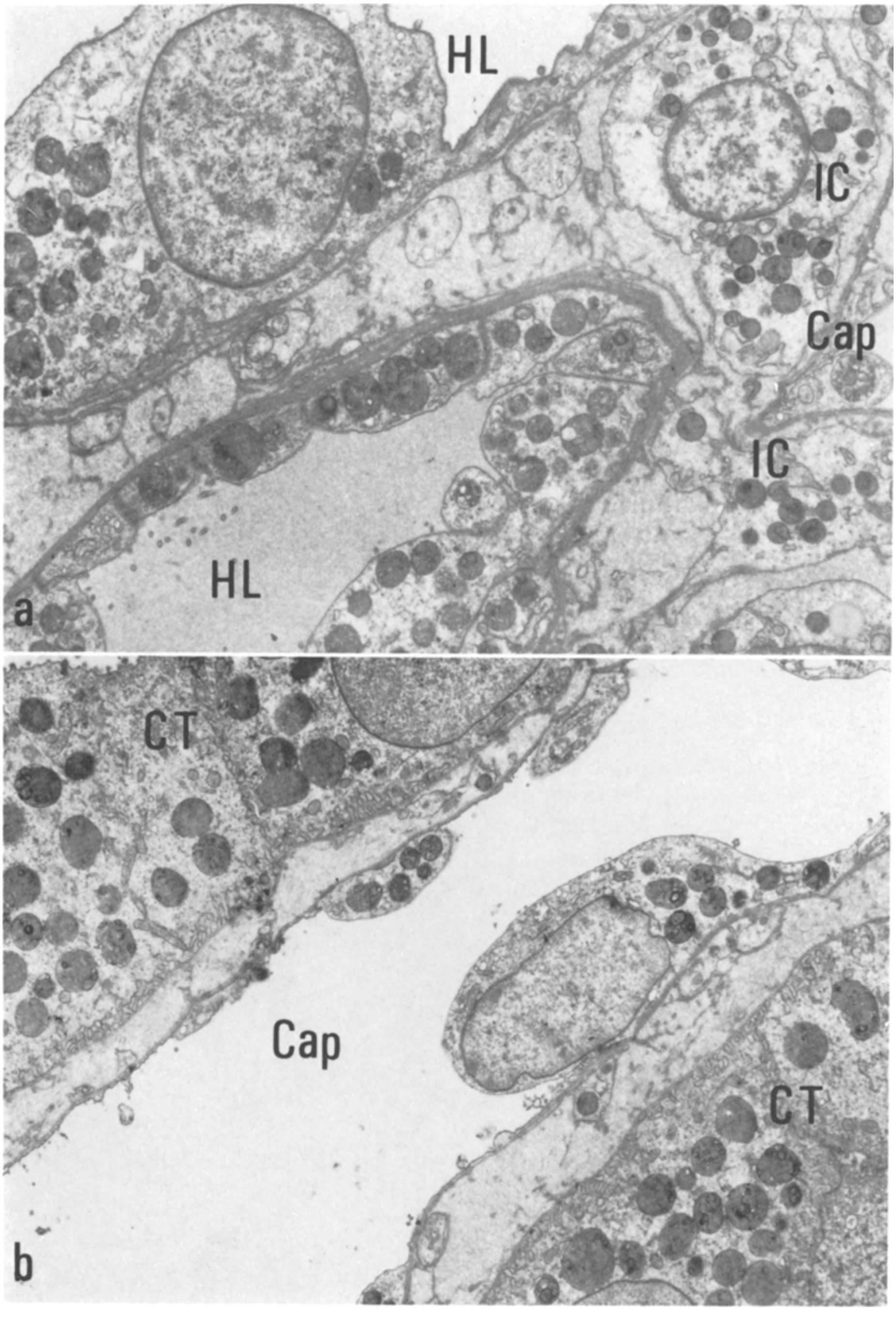

Fig. 7 a, b. Renal inner medulla of one week potassium depleted rat. **a** Granules in Henle's loops (*HL*), interstitial cells (*IC*) and capillary endothelium (*Cap*) (× 5,280) **b** Granules in capillary endothelium (*Cap*) and collecting tubules (*CT*). Granules contain elements similar to those of potassium depleted human kidney and are bounded by a single limiting membrane (× 3,900)

2. Morphological Findings of Kidneys of Potassium Depleted Rats

By light microscopy the renal cortex of potassium depleted rats showed no abnormal findings. After feeding on a potassium deficient diet, accumulation of intracytoplasmic PAS positive granules was invariably present in all cell types located in the inner renal medulla and the papilla. The granular change was most prominent in the epithelium of the collecting tubules and the cells covering the papillary tip. These granules varied in quantity almost in parallel with the osmotic gradient; their number and intensity increased from the inner medulla to the papillary tip. These renomedullary granules were not seen in the kidney of the control rats. Another finding in the kidney of potassium depleted rats was an increase in the number of interstitial cells.

Electron microscopic observations disclosed the unique localization and appearance of intracytoplasmic osmiophilic granules (Fig. 7). The distribution and internal structure of these intracytoplasmic granules were similar to those in previous reports (Spargo et al. 1960; Wilson et al. 1973; Panner 1971; Toback et al. 1976). The granules were bounded by a single limiting membrane and contained small vesicles, amorphous dense materials, vacuoles and lamellar structures. In the renal inner medulla of control rats, a few osmiophilic granules were recognized in epithelial cells and interstitial cells, but their internal structure was more amorphous and easily distinguishable from the intracytoplasmic granules with lamellar structures of the potassium depleted rats. The granules increased in size and number in proportion to the duration of potassium depletion.

Discussion

The prominent renal lesion in the potassium depleted patient with Bartter's syndrome was an accumulation of PAS positive intracytoplasmic granules in the epithelium of collecting tubules, Henle's loops, the interstitial cells, and the endothelium and smooth muscle cells of blood vessels in the inner medulla and papilla. In human cases, tissues from renal biopsy often contain no medulla and renomedullary lesions in potassium depletion have attracted little attention. France et al. (1962, 1973, 1974, 1978) have described renomedullary intracytoplasmic granules in four cases of chronic potassium depletion due to renal potassium wasting. They emphasized that the intracytoplasmic granules appear to be identical to those in potassium depleted rats by light microscopy, though their relation to the osmotic gradient remained uncertain and electron micrographs in one case showed autolytic effects of inadequate fixation. In the present case, these granules appeared in the renal medulla in parallel to the osmotic gradient similar to those observed in the potassium depleted rats. Ultrastructural findings of the granules revealed no appreciable differences between human and rats. But in potassium depleted rats, intracytoplasmic granules were more markedly accumulated in the epithelium of the collecting tubules than any other cell type of the inner medulla. In the human kidney, granular change in renomedullary cells appeared more diffusely in the epithelium of the collecting tubules, interstitial cells and vascular endothelium. This discrepancy may have resulted from the duration and the manner of potassium depletion in two species; whether the onset is acute or chronic, and whether caused by restricted uptake or renal loss of potassium, but the overall features of these intracytoplasmic granules were similar. Verberckmoes et al. (1976) described PAS and silver positive dots of the renomedullary cells in a case with Bartter's syndrome. In their case, accumulation of these granules appeared more prominent in the epithelium of Henle's loops and vascular endothelium rather than collecting tubules and so it remains uncertain whether these granules corresponded with those in the present case. In addition, hyperplasia of the renomedullary interstitial cells has been described in patients with Bartter's syndrome (Erkelens and Statius 1973; Verberckmoes et al. 1976). At present, however, there seems to be no evidence that granular changes of the renomedullary cells are specific to the patients with this unique clinical syndrome.

After treatment with indomethacin, the intracytoplasmic granules showed a notable decrease in their number and intensity of staining with an elevation of the serum potassium concentration. In potassium depleted rats, it has been observed that potassium repletion is followed by a progressive condensation and reduction in the number and the size of intracytoplasmic granules and after three days of potassium repletion, all cells reveal a normal ultrastructural appearance (Ordonez et al. 1977). In patients potassium depleted by chronic diarrhoea, Relman and Schwartz (1956) observed by serial biopsy study that the vacuolar change of the convoluted tubules returned to a normal appearance following the repair of urinary function after replacement of potassium. These findings indicate that morphological changes in the renal tubules are susceptible to the potassium concentration level and reversible at some stages of their development.

Accumulating evidence indicates that the intracytoplasmic granule in potassium depleted rats represents a form of lysosome. Acid phosphatase activity is known to increase in the renal medulla in the course of potassium depletion (Craig and Schwartz 1957; Macpherson et al. 1957; Wachstein and Meisel 1959), and electron histochemical observations have demonstrated the localization of acid phosphatase to granules (Morrison et al. 1963; Panner and Rifkin 1973). The ultrastructural appearance of the granules seen in the human kidney and their parallel distribution with the osmotic gradient strongly suggest the lysosomal nature of these cytoplasmic structures, most probably related to the inability to concentrate the urine, a functional defect in potassium depletion (Milne and Muehrcke 1957; Relman and Schwartz 1958).

It should be noted that the intracytoplasmic granules in the renal inner medulla and the papilla are the characteristic feature of the potassium depleted human kidney and could be regarded as the morphological counterpart of the potassium epleted rat. This finding may have attracted little attention because the accumulation of the intracytoplasmic granules is susceptible to the potassium concentration levels and represents a rapidly reversible change.

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