Brit. J. Pharmacol. (1963), **20**, 352–361.

ULTRASTRUCTURAL CHANGES PRODUCED IN THE RAT KIDNEY BY A MERCURIAL DIURETIC (MERALLURIDE)

BY

A. SANABRIA

From the Laboratorio de Ultraestructura, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela

(*Received January* 23, 1962)

Electron microscopic study of the cortical and medullary zones of kidneys of rats injected intraperitoneally with meralluride (a mercurial diuretic) shows that constant structural changes occur in the proximal tubules, but that the distal tubules remain unaltered. In the cells of the proximal tubule vacuolation and loss of contrast of the apical pole occur due to intracytoplasmic oedema, appearances which may extend as far as the basal pole. The brush border shows separation of the villi at the level of the implantation base. Mitochondrial swelling occurs with vacuolation of the matrix and disappearance of the cristae. The changes at the level of the glomerulus are variable and consist of clearing of the mitochondrial matrix of the podocytes, enlargement of the vesicles of the endoplasmic reticulum and the occurrence of intracytoplasmic osmiophilic masses.

Mercurial diuretics act pharmacologically by inhibiting the capacity of the tubules to reabsorb water and electrolytes, especially sodium and chloride ions (Vogl, 1953). This inhibition is transitory and incomplete and is probably due to a slight, reversible toxic nephrosis without visible microscopic changes, but toxic doses of the diuretics may give rise to evident histological changes (Vogl, 1953). Lesions have been observed with the light microscope at the level of the proximal tubule; with larger doses these lesions can extend as far as Henle's loop (Edwards, 1942). Structural changes in the nephron produced by uranyl nitrate (Bencosme, Stone, Latta & Madden, 1958), by uranyl nitrate, potassium dichromate or mercuric chloride (Mueller & Mason, 1957), and by aminonucleoside (Feldman & Fisher, 1959) have been observed at the submicroscopic level, but none of these drugs is used clinically.

The present work describes an electron microscopical study of the structural changes produced in the rat kidney by the action of a mercurial diuretic injected intraperitoneally. The preparation meralluride (Mercuhydrin by Lakeside), a mixture of *N*-(3-hydroxymercuri-2-methoxypropylcarbamyl) succinamic acid and theophylline, seems to act mainly on the proximal tubule (Wachstein & Meisel, 1954; Vander, Malvin, Wilde & Sullivan, 1958; Kessler, Lozano & Pitts, 1957).

Although meralluride contains the diuretic theophylline, the significant changes are probably due only to the mercury-containing constituent.

METHODS

One hundred and three Sprague-Dawley rats (200 to 250 g body weight) were injected intraperitoneally with meralluride (a solution of the sodium salt containing 39 mg of mercury per ml.) in doses of 0.25, 0.5, 1.0, 2.0 and 4.0 mg. The animals were killed 10, 30, 120 or 240 min after the injection, and their kidneys removed within 1 min. Small pieces were immediately cut from the medullary and cortical zones, fixed in osmium tetroxide by the technique of Rhodin (1954) after dehydration with an ethanol series, and embedded in a

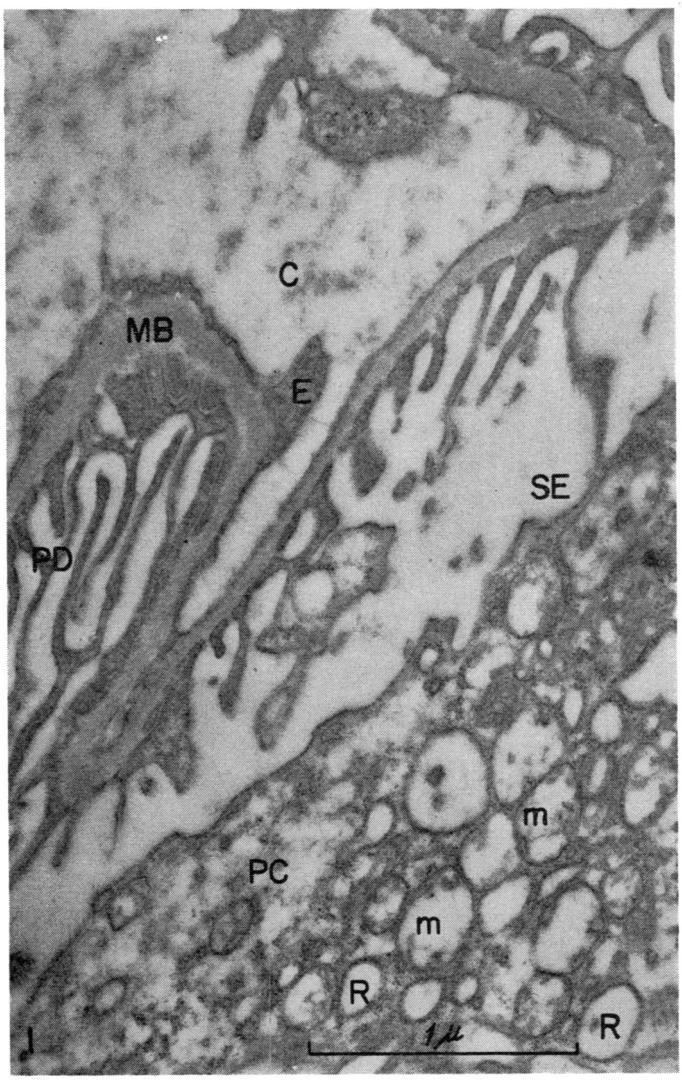

Fig. 1. Electron photomicrograph of the glomerulus from a rat injected with 0·025 ml. of meralluride and killed 1 hr later. On the lower right is a podocyte (PC) showing altered mitochondria (m) in its cytoplasm and widened endoplasmic reticulum vesicles (R). On the upper left is a capillary (C) with its endothelium (E) and basement membrane (MB) unchanged. The pedicels (PD) or foot processes attached to the basement membrane are unchanged. A normal subpodocytic space (SE) separates the podocyte from the capillary.

pre-polymerized mixture of *n*-butyl- and methyl-methacrylate (Borysko, 1956). Ultra-thin sections were cut with a Moran Ultramicrotome equipped with a diamond knife (Fernández-Morán, 1956), and examined with a Siemens electron microscope (Elmiskop I). Numerous control observations were made in order to ensure that the observations described here were not due to artifacts caused by the fixation or embedding media.

RESULTS

After intraperitoneal injection of meralluride at increasing doses into adult rats, submicroscopic changes occur, especially in the proximal tubule. They can be

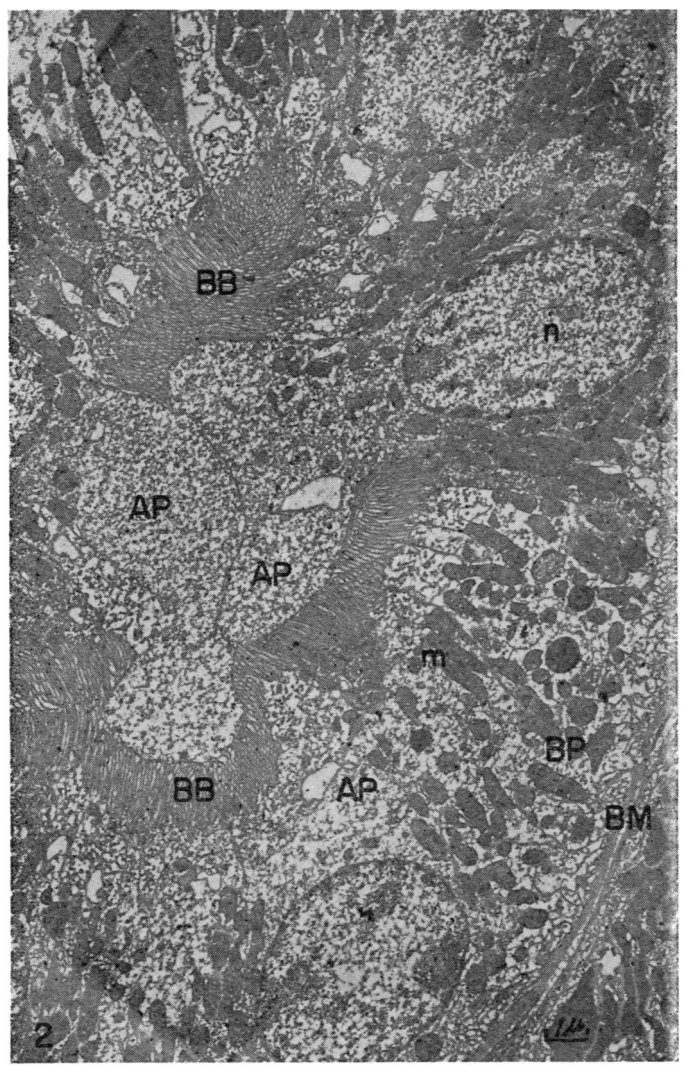

Fig. 2. Electron photomicrograph of a proximal tubule from a kidney of a rat injected with 0·1 ml. of meralluride and killed 10 min later. The cytoplasm of the apical pole (AP), under the brush border (BB), shows a clear matrix, while the basal pole (BP), the nucleus (n), the mitochondria (m) and the basement membrane (BM) are normal.

observed 10 min after the injection, and reach their maximum after 30 min ; 240 min after the injection they are still present.

The morphological changes in each segment of nephron will be described separately.

Glomerular changes

Glomerular changes were not constant. Occasionally they were observed at the level of the podocytes in which clearings of the mitochondrial matrix occurred ; disappearance of the cristae and enlargement of vesicles in the endoplasmic

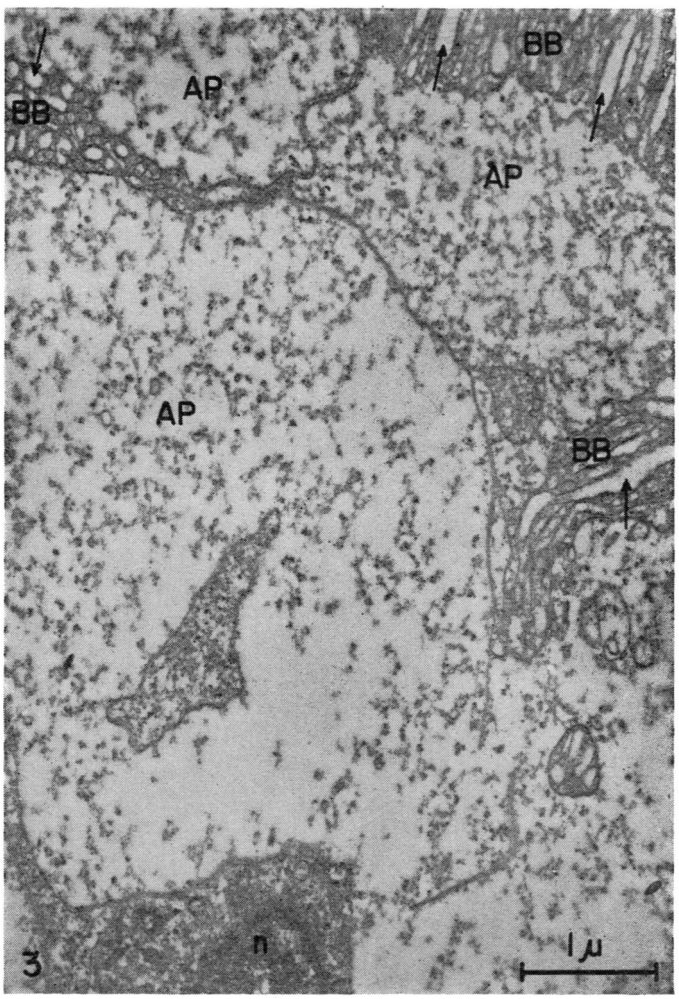

Fig. 3. Electron photomicrograph of the apical pole (AP) of the cells of a proximal tubule of a rat injected with 0.0062 ml. of meralluride and killed 30 min later. The cytoplasm shows a clear matrix filled with many fine granules approximately 150 Å in diameter. Portions of the brush border (BB) show a widening of the spaces between the villi (arrows). Other abbreviations as in Figs. 1 and 2.

reticulum were also observed (Fig. 1). No changes were apparent in the pedicels or foot-processes, or in the dense and fenestrated plates.

Changes of the proximal tubule

Constant changes appeared in proximal tubules of material obtained 10 min after the intraperitoneal injection of meralluride in high doses (for example, 4.0 mg; Fig. 2), and after 30 min in the material obtained after the injection of 0.25 mg

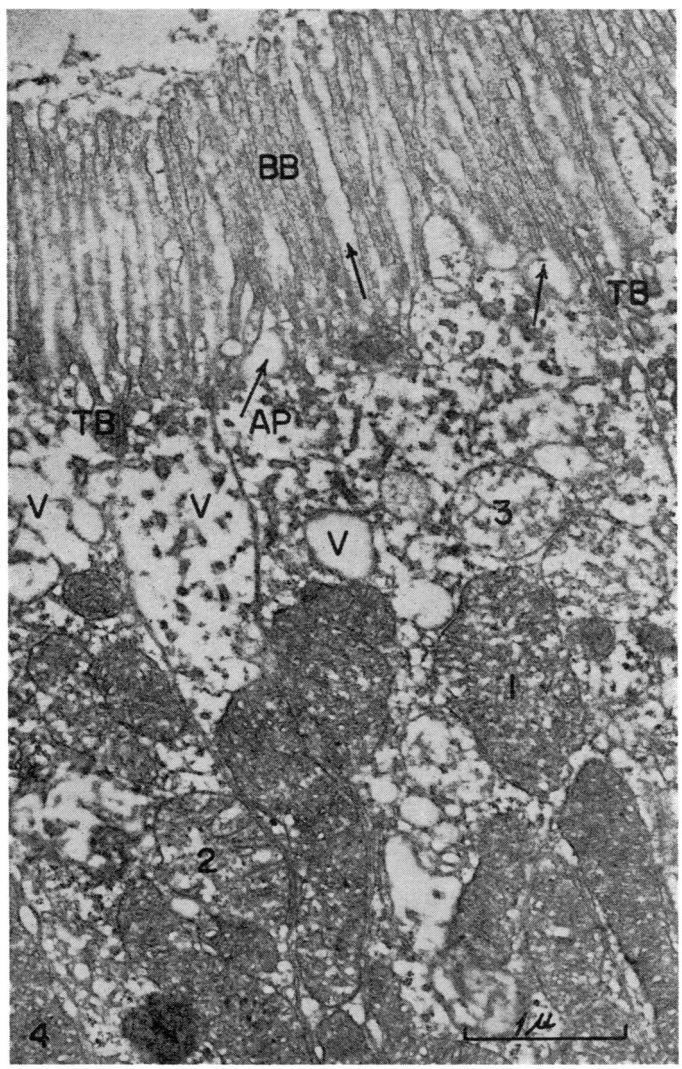

Fig. 4. Electron photomicrograph of the apical pole (AP) of a proximal tubule of a rat injected with 0.1 ml. of meralluride and killed 3 hr later. The brush border (BB) shows a separation of the villi (arrows). Vacuolation of the cytoplasm is seen in several places (V). At 1, 2 and 3 the pattern of the mitochondria is altered. Terminal bars (TB) are seen at the intercellular limits.

(Fig. 3). As these structural changes are different in each of the tubular segments, they will be described separately.

Changes in the brush border. These consist of a separation of the villi at the level of their implantation base which is generally apparent after 30 min, with various doses of meralluride (Figs. 3 and 4). With high doses the separation of the villi is more evident (Fig. 4), and occasionally all the structures disappear.

Cytoplasmic changes. In the apical pole of the proximal tubule, immediately below the brush border, there are zones of diminished electron density which some-

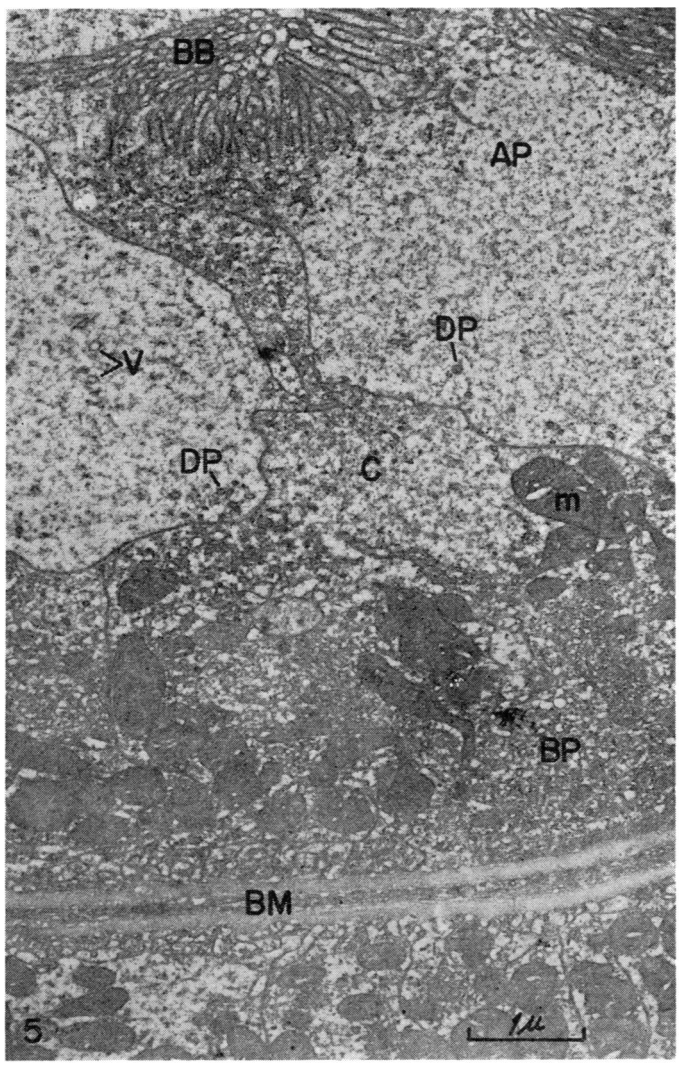

Fig. 5. Electron photomicrograph of a proximal tubule of a rat injected with 0.1 ml. of meralluride and killed 30 min later. The apical poles (AP) of three of the cells show the changes described for Fig. 3. Small dense granules (DP) and a few vesicles (V) can be seen scattered among the fine granules which fill the clear cytoplasm. At C, the apical poles of two cells show no changes.

times spread out as far as the basal pole and appear to be due to intracytoplasmic oedema (Figs. 2, 3 and 5). The diminished density does not occur in all the cells of the same tubule (Fig. 5) but is the most common change at the level of the proximal tubule; it can be observed with all doses of meralluride, usually 30 min after injection although I have seen it after 10 min with doses of 4.0 mg. The zones of diminished electron density contain numerous small granules 150 Å in diameter, while a few small vesicles and granules of larger diameter (Fig. 5) and aggregations of large vacuoles were also seen (Fig. 4).

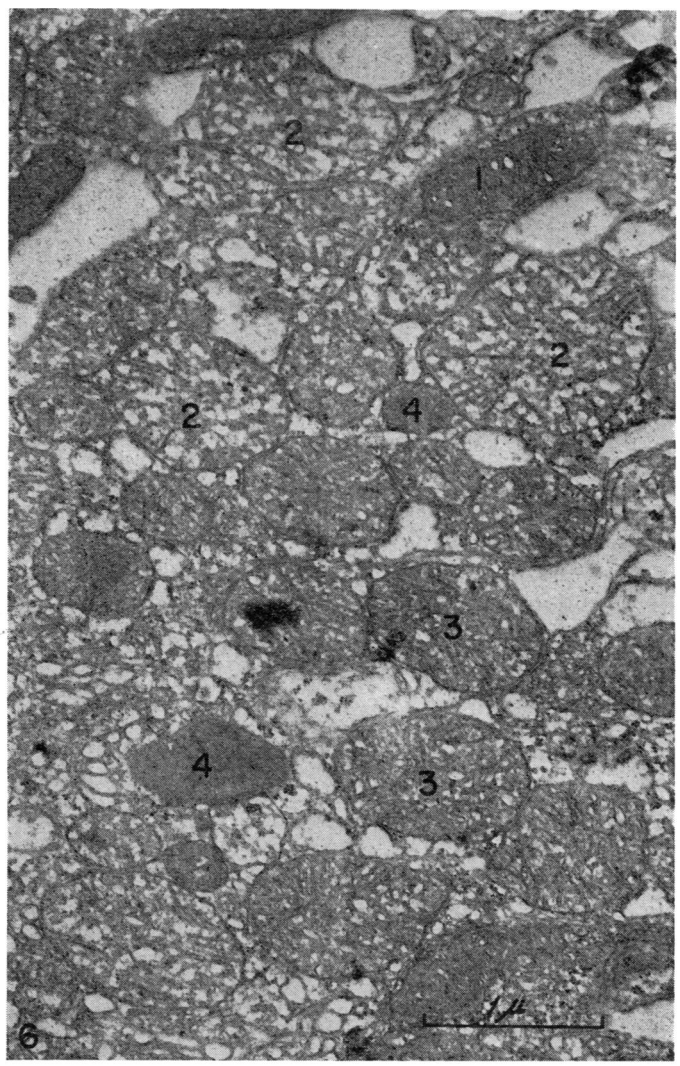

Fig. 6. Electron photomicrograph showing the basal pole of a proximal tubule of a rat injected with 0.025 ml. of meralluride and killed 2 hr later. A normal mitochondrion is seen at 1. The rest of the mitochondria appear swollen. At 2, the matrix is clear and contains vacuoles, and the cristae are fragmented, while at 3, the matrix is dense. Dense rounded structures without cristae but with the same mitochondrial pattern are seen at 4.

Mitochondrial changes. These are frequent but do not affect equally all the mitochondria of one cell (Figs. 6 and 7); usually they begin 30 min after injection and are independent of the dose administered. They consist of a swelling of the mitochondrial matrix and disappearance of the cristae; the cristae may be reduced to small segments linked by the internal mitochondrial membrane (Figs. 4 and 6). The clearing of the mitochondrial matrix is due to the formation of small vacuoles which become confluent. In some cells, with higher doses, the vacuolation is complete, the mitochondria being reduced to their external membranes with only remnants of cristae, as had been observed after injection of mercuric chloride;

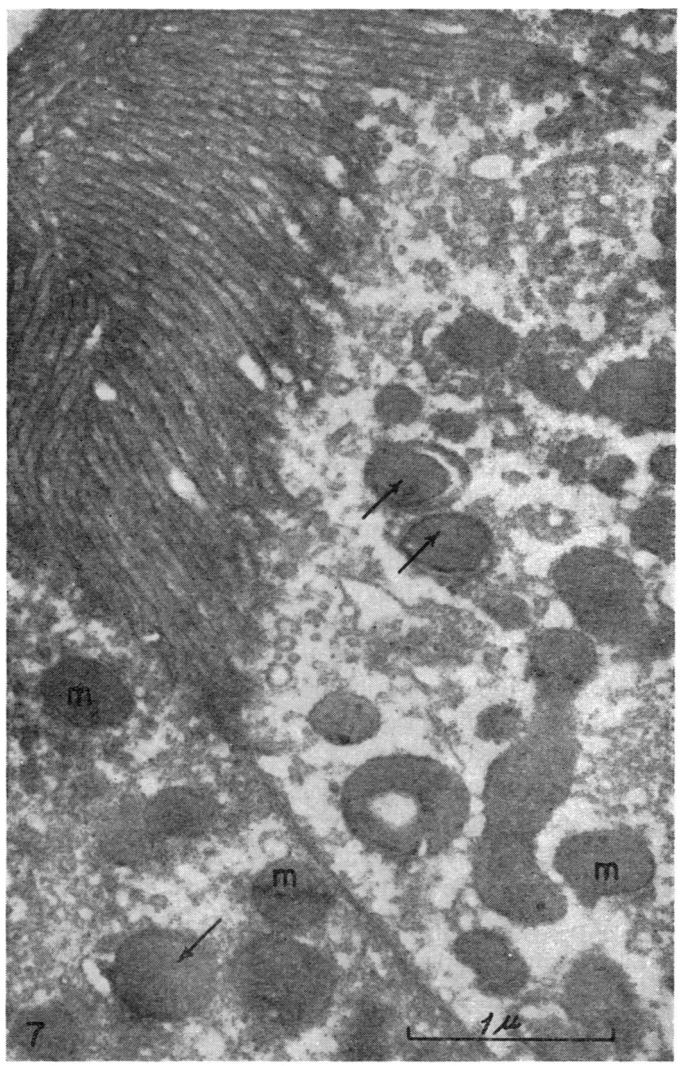

Fig. 7. Electron photomicrograph of a proximal tubule of a rat injected with 0·05 ml. of meralluride and killed 3 hr later. Normal mitochondria (m) and dense bodies surrounded by membranes but without cristae (arrows) are shown at the apical poles of the cells.

this appearance was described by Mueller & Mason (1957) as the formation of a "mitochondrial phantom." Similar structures, dense internally and without cristae, were also observed (Fig. 6). These dense bodies, surrounded by a membrane, appear in large numbers toward the apical pole (Fig. 7).

Distal tubules, collecting tubules and Henle's loop

In these experiments no structural changes were observed in these parts of the renal tubule.

DISCUSSION

The study of the submicroscopic changes after intraperitoneal injection of meralluride supports a number of conclusions that have already been reached about the pharmacology of mercurial diuretics. They cause changes, mainly in the proximal tubules, which vary with the dose used and with the time after dosage, but they do not affect the rest of the kidney.

Changes in the proximal tubule appear 10 min after intraperitoneal injection and reach their maximum after 30 min with doses of 0.25 mg. With higher doses, they become very intense and, in some instances, may cause a complete structural disorganization of the tubule.

The ultramicroscopic appearance after using meralluride consists of changes which are frequent at the level of the proximal tubule, but rare and various at the glomerular level; they are similar to those observed when mercuric chloride was used (Mueller & Mason, 1957). This appearance is consistent with the suggestion of Brun, Hilden & Raaschau (1947) that mercury is actively excreted by the tubular cells where it attains its maximum concentration. The mercury apparently acts on the enzymatic system responsible for the reabsorption of sodium, of chloride and of water; this effect on the tubular cells may be due to the mercury becoming attached to the plasma proteins so that small quantities of diuretic penetrate into the glomerular filtrate (Kessler *et al.*, 1957; Borghgraef, Kessler & Pitts, 1956). This conclusion is supported by autoradiographic studies of isolated nephrons by Darmady & Stranack (quoted by Hess, 1959), which show a preferential accumulation of mercury in the proximal tubule, and in addition the histochemical study by Hess (1959) shows that mercurial diuretics change the enzymatic mitochondrial activity, most obviously in the proximal tubules.

Diminished contrast due to intracytoplasmic oedema is the most constant morphological change, this effect being due to the slower absorption of water and solutes in the proximal tubule, and by the temporary disturbances in the energy control mechanism which is responsible for reabsorption.

The author thanks Drs G. M. Villegas and T. Imaeda for their valuable suggestions, and J. Suter and Zuleika Gorrin for technical assistance.

REFERENCES

BENCOSME, S., STONE, R. S., LATTA, H. & MADDEN, S. C. (1958). Acute renal lesions produced by uranium nitrate. *Proc. Ann. Meeting of the Electron Microscopic Soc. of Amer.*, Santa Monica, California, p. 6.

BORGHGRAEF, R. R. M., KESSLER, R. H. & PITTS, R. F. (1956). Plasma regression, distribution and excretion of radiomercury in relation to diuresis following the intravenous administration of Hg^{203} labelled chlormerodrin to the dog. *J. clin. Invest.*, **35**, 1055–1066.

BORYSKO, E. (1956). Recent developments in methacrylate embedding. I. A study of the polymerization damage phenomenon by phase contrast microscopy. *J. biophys. biochem. Cytol.*, **2** (suppl.), 3–14.

BRUN, C., HILDEN, T. & RAASCHAU, F. (1947). On the effects of mersalyl on the renal function. *Acta pharmacol. (Kbh.)*, **3**, 1–12.

EDWARDS, J. G. (1942). The renal tubule (nephron) as affected by mercury. *Amer. J. Path.*, **18**, 1011–1027.

FELDMAN, J. D. & FISHER, E. R. (1959). Renal lesions of aminonucleoside nephrosis as revealed by electron microscopy. *Lab. Invest.*, **8**, 371–385.

FERNÁNDEZ-MORÁN, H. (1956). Applications of a diamond knife for ultrathin sectioning to the study of the fine structure of biological tissues and metals. *J. biophys. biochem. Cytol.*, **2** (suppl.), 29–30.

HESS, R. (1959). Die Histochemische Analyse enzimatischer vorgange in Nierentubulus. In *Diurese und Diuretica*, p. 121. Berlin: Springer-Verlag.

KESSLER, R. H., LOZANO, R. & PITTS, R. F. (1957). Studies on structure diuretic activity relationships of compounds of mercury. *J. clin. Invest.*, **36**, 656–668.

MUELLER, C. B. & MASON, A. D., JR. (1957). A study of the pathologic anatomy of the proximal tubules of the mouse produced by various nephrotoxins. *Surg. Forum*, **7**, 641–645.

RHODIN, J. (1954). *Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convolute Tubule Cells of the Mouse Kidney.* Stockholm: Karolinska Institute.

VANDER, A. J., MALVIN, R. L., WILDE, W. S. & SULLIVAN, L. P. (1958). Localization of the site of action of mercurial diuretics by stop-flow analysis. *Amer. J. Physiol.*, **195**, 558–562.

VOGL, A. (1953). *Diuretic Therapy.* Baltimore: Williams & Wilkins.

WACHSTEIN, M. & MEISEL, E. (1954). On the histochemical localization of the mercurial inhibition of succinic dehydrogenase in rat kidney. *Science*, **119**, 100.