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Histological and Ultrastructural Changes in Rat Kidney Following Cadmium Injection

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Summary. Cadmium injection in rats affects the genito-urinary system. The renal effects are generally considered to be mainly tubular. Light microscopy of 29 specimens confirms cellular damage in the glomeruli with changes in glomerular capillaries. Electron microscopic changes in the glomeruli confirm changes in the podocytes, thickening of the basement lamina, the occurrence of electron dense deposits in association with the capillary endothelium with platelet and polymorphonuclear cell accumulations which suggests opening of the endothelial junction of the glomerular vessels.

Key words: Cadmium - Kidney - Ultrastructure.

Injection of cadmium subcutaneously in the experimental animal causes an initial testicular oedema followed by destruction of the seminiferous epithelium; in the long term interstitial cell tumours occur (21, 20). Subcutaneous sarcomata develop at the site of injection in the experimental animal (13). In addition to the effects on the genitalia, injection of cadmium produces renal damage (2, 3, 24, 4, 5). The present experiment describes the microscopic and ultrastructural effects of cadmium on glomeruli and tubular cells.

MATERIAL AND METHODS

50 Wistar rats were injected subcutaneously in the shoulder area with 0.5 ml of a sterile cadmium chloride solution, equivalent to 0.17 mg cadmium/kg as a free ion. Repeat injections were made in batches of 10 animals over a 6 month period up to a maximum of 5 injections in any one group. The main aim of the experiment was to determine the prostatic effects of these injections. Following the last injection the rats received no further injections until either they developed subcutaneous tumours or showed obvious evidence of becoming ill at which time they were sacrificed.

During the course of the experiment 5 animals developed a sudden fatal pneumonia and were excluded from the study.

The blocks for renal histology and the specimens for ultrastructure were obtained by removing the lower pole of the left kidney while the animal was anaesthetized prior to sacrifice.

Twenty-nine partial nephrectomy specimens were obtained for light and ultrastructural examination.

Light Microscopy

Blocks of tissue from the lower pole of the left kidney were fixed in Zenker-formol. Sections of 5 μthickness were cut and stained with haematoxylin and eosin and periodic acid-Schiff.

Electron Microscopy

Pieces of tissue were fixed in 2% glutaraldehyde (23) post-osmicated, dehydrated and embedded in epon (17). Fine sections mounted on copper

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Table 1. Post-mortem lesions in cadmium injected rats

	Group 1 9 rats	Group 2 9 rats	Group 3 10 rats	Group 4 5 rats	Group 5 10 rats
Bilateral testicular atrophy	7	4	4	3	5
Unilateral testicular atrophy	-	1	-	-	-
Testicular atrophy with necrosis	1	3	6	1	4
Testicular cyst	1	-	-	-	-
Enlarged testis	-	-	1	-	-
Enlarged prostate	-	-	1	-	-
Subcutaneous tumour	-	4	3	4	6
Intraperitoneal tumour	-	1	-	-	-
Diffuse carcinomatosis		-	-	-	1
Pneumonia	-	-	1	-	-
Pale nodular liver	-	-	1	-	-
Liver worm	-	-	-	-	1

grids were stained with uranyl acetate and lead citrate and examined in a Hitachi HS8 electron microscope.

RESULTS

Gross Findings in 45 Animals

These are summarized in Table 1. No animal which received only one injection of cadmium chloride developed a subcutaneous tumour. All animals had microscopic evidence of testicular damage and in 23 there was macroscopic atrophy of the testes. In one animal there was, surprisingly, unilateral atrophy of the testis. In 15 animals the subcutaneous tumours were shown to be fibrosarcomata and in 1 animal there was diffuse local spread of tumour. One animal had fibrosarcoma of the stomach with no evidence of a subcutaneous lesion.

With increasing dosage one would have expected more pathology in each group but surprisingly those receiving 4 injections had less pathology than those with 3 injections, and in 34 animals where the weight was taken at post mortem, those receiving 5 injections had a greater overall body weight than those with a lower total dosage of cadmium (Fig. 1). There is no explanation for this phenomenon.

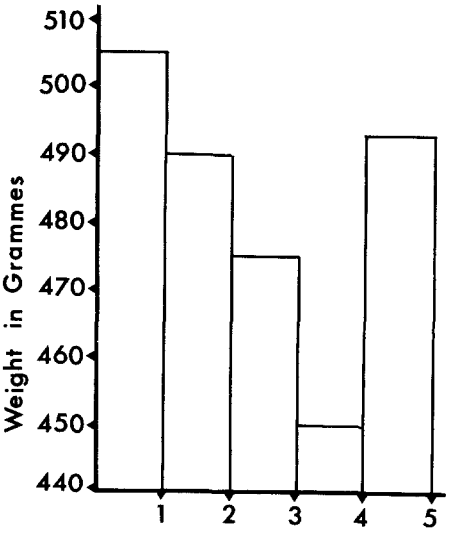

Fig. 1. Weight of rats at postmorten. Via the number of cadmium injections

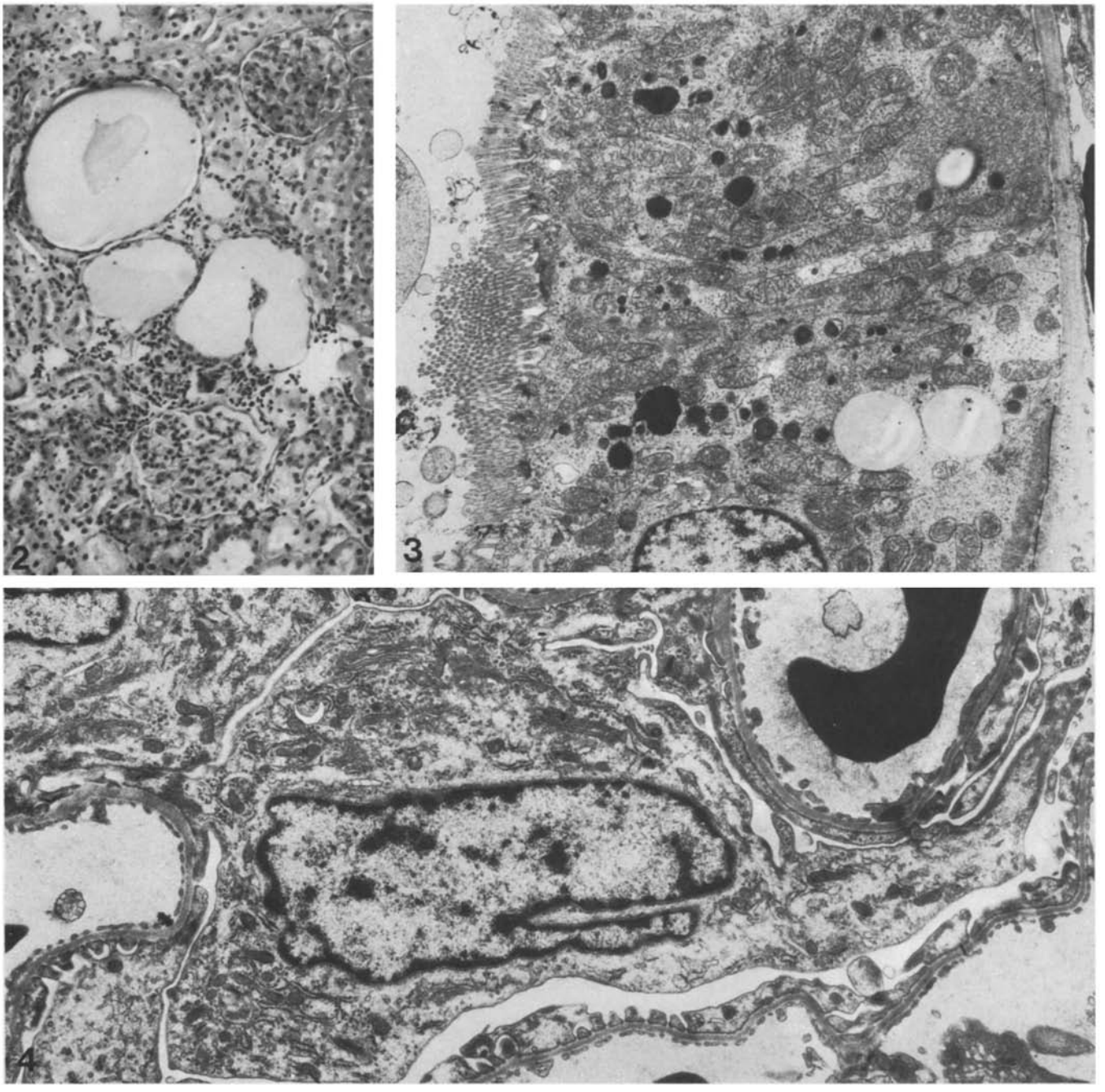

Fig. 2. Low power micrograph of rat renal cortex to show the distended tubules and a distorted glomerulus with a thickened basement membrane. H/PAS x 100

Fig. 3. The proximal convoluted tubule showing reduction in the height and complexity of microvilli along the lumenal border and loss of basal infoldings. The basal lamina is thickened. Uranyl acetate/lead citrate x 4 500

Fig. 4. The glomerular epithelial cell illustrated here shows the extensive complex nature of the cytoplasmic organelles and some withdrawal of foot processes. Uranyl acetate/lead citrate x 8 000

HISTOLOGY OF THE KIDNEY

Light Microscopy

The kidneys of cadmium treated rats showed considerable tubular degeneration and glomerular damage (Fig. 2). Areas of desquamation and damage of tubular cells were evident in the irregularly outlined tubules, some of which were filled with an amorphous material. These latter tubules were commonly dilated and lined by a cuboidal or even squamous epithelium. The degree of damage includes haemorrhage, fibrosis and cellular infiltration and these effects were most evident in the high cadmium dosage animals. An increase in basement membrane occurs, as shown by the periodic acid-Schiff reaction, and an increased cellularity of individual glomeruli. No increase was evident in the parietal layer, but there were areas of fusion between this layer and the glomerular capillaries.

Electron Microscopy

In the glomerulus the podocytes appeared as large cells with extensive cytoplasm, a well developed Golgi apparatus and associated smooth membranes, small areas of rough endoplasmic reticulum (R.E.R.) and free ribosomes (Fig. 3). Many foot processes appeared to be fused into one large blocking process. The basal lamina was noticeably thicker in some sections.

Electron dense deposits were rarely evident in the basal lamina between the epithelial cell and the endothelium of the glomerular capillary. Intracellular dense fibrillar deposits were also present in the epithelial cell, the nucleus of which was commonly distorted. Some degree of swelling of the epithelial cells was also apparent in some areas and large membrane bound swollen cell fragments were commonly seen in the urinary space. These were usually electron lucent and had free ribosomes scattered throughout, though complex membrane patterns were also observed. In some cells the filtration slits were close to open channels of rough endoplasmic reticulum.

The mesangial cells were found singly or in groups and had many organelles including a Golgi apparatus, smooth endoplasmic reticulum with small vesicles, rough endoplasmic reticulum, free ribosomes and dense bodies, possibly lysosomes. Dense ground substance was a feature of the mesangium in these glomeruli.

The parietal cell basement membrane was massively thickened, and electron dense deposits were a common finding. The general appearance of the membrane was fibrillar. Platelets and polymorphonuclear leucocytes were a common finding in these glomeruli. The lumen of the proximal convoluted tubule was filled with electron dense granular detritus, some of which appeared to be membrane bound. The microvilli showed some blebbing, and the absorptive zone between the bases of individual microvilli contained vesicles with some contained particulate material (Fig. 4). There was a general increase in dense bodies with the lining cell of the proximal convoluted tubule showing a reduction in height of the cell and loss of the basal infolding of the plasma membrane. The interstitium consisted of active fibroblasts, collagen fibres and dense accumulations of calcified deposits within amorphous material identical to that of the basement membrane.

The cellular changes described above were not so pronounced in the distal convoluted tubule, but there was an increase in the dense bodies and often an obvious thickening of the basement membrane and the appearance of electron dense deposits.

DISCUSSION

In a previous experiment the present authors described the histological changes in the rat prostate gland following the direct injection of cadmium into either the ventral or the dorsolateral lobe (25). When these animals were examined at post mortem renal lesions were observed; macroscopic due to back pressure effects and microscopic as a possible results of cadmium accumulation in the kidney (1, 12). As expected subcutaneous tumours occurred at the site of injection (14) and the testes in most animals were found to be atrophic or necrotic (21).

Cadmium nephropathy in the experimental animal is typified by tubular damage and glomerular amyloidosis (2, 3, 7, 24). The kidney cortex selectively accumulates cadmium (1, 12) where it is associated with a low molecular weight protein, cadmium thionein (15, 16, 18). In a recent study cadmium bound thionein was shown to be more nephrotoxic than cadmium chloride (19). These authors suggested that the cadmium protein is filtered through the glomeruli and reabsorbed and stored by the renal tubules. Most authors regard renal tubular damage as being the single most important characteristic of cadmium nephropathy (10). The present authors however consider the glomerular lesions to be equally important. A recent report has shown that injection of cadmium in rats causes a rise in plasma renin activity with hypertension (22)

and this emphasises that the glomerular effects described in this experiment are possibly of greater significance than was previously recognized.

In this particular experiment the significance of the glomerular lesions are emphasised in view of the demonstration of a high molecular weight protein in the urine of cadmium workers (26). Previous studies have tended to emphasise low molecular weight proteinuria of tubular origin (8-10).

The absence of regular deposits in the basal lamina negate the possibility of a glomerulonephritis, confirming the work by Bonnell et al. (3) who compared the cadmium induced renal lesions to those found in pyelonephritis but were unable to culture any organisms from these kidneys.

The changes described in the glomerular epithelial cells were more closely allied to those described in renal toxicity states (6) where the withdrawal of foot processes and degenerative changes in the epithelial cells were described. This also gives rise to a proteinuria. Of course, any disruption of renal function in the epithelial cell would be reflected in the basal lamina, the filtration system of the glomerulus.

In addition, it has recently been shown that cadmium opens the tight endothelial junctions in testicular vessels (11) and it is possible that the disruption of the capillary endothelium would aggravate the filtration problem induced by malfunction of the glomerular epithelial cells. Further evidence of endothelial cell disruption is provided here by the obvious accumulation of platelets in the glomerular capillaries. This may be a direct response to cadmium.

This report shows the importance of the glomerular lesion in cadmium toxicity and reinforces the need to understand the mechanism by which this occurs, and the relationship between proteinuria in the experimental animal and that in the worker exposed to cadmium over a period of time.

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