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Morphological Effects of Cadmium on Proximal Tubular Cells in Rats

KIYOSHI MATSUURA,[1] MASAYUKI TAKASUGI,*,[1]
YASUMASA KUNIFUJI,[2] AKIO HORIE,[3] AND AKIO KUROIWA[1]

[1]The Second Department of Internal Medicine, School of Medicine; [2]Division of Clinical Chemistry, School of Medical Technology; and [3]Department of Pathology, and Oncology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807, Japan

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ABSTRACT

Twenty-four male rats of the Wistar strain divided into four groups were injected sc with a dose of 0.8, 1.5, and 3.0 mg Cd/kg body wt as $CdCl_2$ in saline, and saline alone to the control rats, three times a week for 3 wk. Cadmium levels of whole kidney homogenate, supernatant (cytosol), precipitate, and metallothionein (MT) fraction were measured. Histological changes of the renal proximal tubules were investigated by optical and electron microscopy. In the kidneys, Cd levels were increased with the increment of Cd dosage; 80–90% of Cd was contained in cytosol, and 55–75% was in MT fraction. Non-MT-Cd reached a maximum in the 1.5 mg Cd group, whereas that of the 3.0 mg Cd group showed some decline. With increasing Cd doses, the size of nuclei and nucleoli in the cells of proximal tubule showed significant enlargement and also an increase in the number of nucleoli on light microscopy. At higher doses, chromatin condensation of the tubular nuclei and vacuolar degeneration of the tubular cells were evident.

On electron microscopy, perichromatin granules of the proximal tubular nuclei were increased in number, especially in the rats of Cd 0.8 mg and 1.5 mg/kg groups. As the Cd doses increased, ring-shaped nucleoli were increased in number and nucleolar segregation

*Author to whom all correspondence and reprint requests should be addressed.

was observed more clearly. Moreover, in the 3.0 mg/kg Cd group, nuclear indentation and nucleoli containing compact dense granules were observed. In the cytoplasm, there was an increase of lysosomes, myelin bodies, ring-shaped mitochondria, and vesiculation; ultimate changes were degeneration and cell necrosis. The injured cells were heterogenously distributed in each nephron and this heterogeneity was attributed in the difference in Cd content and cell cycle in each cell of the nephron.

Index Entries: Cd; kidney; tubular nucleus; light microscopy; electron microscopy.

INTRODUCTION

It is well known that cadmium (Cd) causes renal damage in humans and in experimental animals (*1*). To elucidate the mechanisms of Cd toxicity, numerous intracellular effects of cadmium have been under extensive in vitro studies using a variety of cells. These include inhibition of DNA, RNA, and protein synthesis (*2–4*), modification of carbohydrate metabolism (*5*), change in the content of microsomal cytochromes (*6*), and an induction of metallothionein (MT) (*7*). Cd-MT was mainly induced by liver and kidney and was reported to be a detoxifying factor (*8*).

These cellular effects may result in various structural derangements. In the present paper, we report experiments demonstrating a structural effect of Cd on renal proximal tubules in the Wistar male rats following the subcutaneous injection of repeated low doses of Cd.

MATERIALS AND METHODS

Animals consisted of male rats of the Wistar strain at 4 mo of age weighing from 250 to 310 g. Animals were divided into four groups, and they were injected sc with 0.8, 1.5, and 3.0 mg Cd/kg as cadmium chloride in saline, three times a week for 3 wk. The control group was injected saline sc in the same manner. Each group consisted of six rats, and was kept in stainless steel cages with free access to drinking tap water and standard rat chaw (CLEA & Co, Tokyo, Japan).

Experimental animals were exsanguinated 48 h after the last injection. Then the kidneys, testes, liver, and adrenals were removed and weighed. Kidneys were homogenized and the homogenate was centrifuged at 170,000*g* (4°C) for 60 min. The homogenate and its supernatant and precipitate were wet-ashed, and the Cd concentrations of the digests were measured with atomic absorption spectrophotometer (Perkin-Elmer 603, flame, Beaconfield, UK). A portion of kidney supernatant was applied to an HPLC gel filtration column and the MT fraction was collected. Cd concentrations of the MT fractions were measured using an atomic absorption spectrophotometer (Hitachi 170-70, flameless, Tokyo, Japan). Detailed analytical procedures were reported elsewhere (*9*).

For optical microscopic examination, a part of each organ was fixed in 10% formalin, embedded in paraffin, sectioned at 3 μm in thickness, and stained with H&E, PAS, Azan, and the Feulgen reaction.

For the study of nuclear morphological changes, nuclear diameters of 50 nuclei were measured using a microcaliper. In each nucleus, the mean of long and short axes of the nucleus was defined as the diameter of a given nucleus. For the study of nucleoli, 60 nuclei in the proximal tubules in five rats from each group were examined under optical microscopy. Nucleolar changes were graded from 0 to IV according to nucleolar findings (0: no nucleolus in proximal tubular nucleus, I: small nucleolus, II: intermediate nucleolus, III: large nucleolus, IV: two nucleolei or more). The incidence of each nucleolar grade in each group was averaged and expressed as a mean. The statistical analysis was performed by Student's *t*-test.

For the electron microscopic study, thin razor slices of left renal cortex 2 × 1 × 1 mm were removed and fixed instantaneously in cold Karnovsky solution. The tissue was then postfixed in 2% osmium tetroxide, dehydrated in a series of graded alcohol, and embedded in Epon 812. Sections cut with diamond knives were double stained with uranyl nitrate and lead citrate and examined with a Japan Electronics JEM 100 c electron microscope.

RESULTS

The average body and organ wt are presented in Table 1. Growth of Cd-treated rats was significantly retarded as the dose of $CdCl_2$ increased. However, the wt of the liver and the kidneys were not significantly different in each group. The testes in the Cd group were decreased in wt, and were whitish in appearance, demonstrating moderate and severe atrophy in the 1.5 and 3.0 mg Cd/kg groups.

The adrenals tended to be enlarged in the 3.0 mg/kg Cd group, but their average wt were not statistically different in each group. Cadmium levels in kidney are summarized in Table 2. The increase in the Cd concentrations of the whole kidneys and MT fractions was directly proportional to the dosage of Cd. As for the Cd concentrations in non-MT fractions, the 1.5 mg Cd group showed maximum levels, whereas the 3.0 mg Cd group contained much less non-MT Cd.

Light microscopy revealed changes in the renal proximal tubules of rats injected with $CdCl_2$, such as enlargement of nuclei and nucleoli in size, and an increase in number of nucleoli, as shown in Figs. 1 and 2. With increasing Cd doses, a progressively higher degree of nuclear and nucleolar enlargement, as well as an increase in the number of nucleoli were observed. At higher doses, chromatic condensation was evident, and in the Cd 3.0 mg/kg group, chromatin dispersion and vacuolar

Table 1
Body Wt and Organ Wt of the Control and Experimental Rats

Treatment Group	n	Body wt, g		Organ, g			
		Before Cd administration	After 3-wk Cd injection	Liver	Kidneys	Testes	Adrenals
Control	6	283.3 ± 17.3	364.5 ± 21.2	11.6 ± 1.1	2.4 ± 0.2	3.1 ± 0.1	0.05 ± 0.01
0.8 mg Cd/Kg	6	291.8 ± 10.6	349.7 ± 11.1	13.2 ± 1.3	2.4 ± 0.2	3.0 ± 0.1	0.05 ± 0.01
1.5 mg Cd/Kg	6	282.7 ± 19.8	300.1* ± 20.6	11.2 ± 1.5	2.4 ± 0.3	1.0** ± 0.1	0.05 ± 0.01
3.0 mg Cd/Kg	6	283.5 ± 17.6	255.3* ± 26.8	11.3 ± 0.8	2.3 ± 0.2	1.0** ± 0.1	0.07 ± 0.22

Significantly different from the control: $^{*}p < 0.05$; $^{**}p < 0.01$ (Mean ± SD).

Table 2
Cd Concentration (μg Cd/g Wet Tissue) in Kidneys from the Control and Cd-Treated Rats

		mg Dose group		
	Cont	0.8	1.5	3.0
Ppt-Cd	N.D.	14.3	24.1	27.6
non-Mt-Cd	N.D.	27.8	44.6	30.2
MT-Cd	N.D.	24.8	55.3	94.3
Total-Cd	N.D.	64.3	119.7	145.2

Values are averages from two pool samples (each sample consists of three rat kidneys).
N.D.: nondetectable.
Ppt-Cd: Cd in precipitable.
MT-Cd: Cd in metallothionein fraction.
non-MT-Cd: computed as difference between supernatant Cd and MT-Cd.

degeneration of the proximal tubular cells were observed in a small number of cells.

By electron microscopy, the perichromatin granules, namely, the electron-dense, solitary, spherical granules separated from adjacent chromatin by an electron-lucent halo, were markedly increased in number at the periphery of nucleolus in the 0.8 and 1.5 mg/kg Cd groups (Fig. 3). In the 3.0 mg/kg Cd group, however, the increase in number of perichromatin granules was not so prominent and only a slight increase was observed in comparison to the control group. Ring-shaped nucleoli with electron-dense rings of nucleolar material enclosing less intensely staining central zones (*see* Fig. 4) increased in number with Cd dosage and segregation of the nucleolar material, the discrete grouping of fibrillar and granular components of nucleolar material into zones of differential electron density were more clearly observable. Moreover, some of these nucleoli had dense granules in the nucleolar material (Fig. 5). In the 3 mg/kg Cd group, nuclear indentation (Fig. 4), pseudoinclusion bodies, and compacted nucleoli (Fig. 7) were observed, however, no true nuclear inclusion bodies were found. Many nuclei in the 3.0 mg/kg showed pyknosis; however, there were also some nuclei that showed mostly dispersed chromatin with decreased heterochromatin (i.e., increase of euchromatin).

In cytoplasm lysosomes, myelin bodies and giant myelin bodies increased with increase in Cd dosage. In rare cases, lysosome-like materials contained dense particles, and double membranes were observed in the Cd 3.0 mg/kg group (Fig. 8). Some mitochondria showed distortion, and some of them were ring-shaped (Fig. 9), especially in the 3.0 mg/kg Cd group. With progressive degeneration, the cytoplasms of proximal tubular cells showed vacuolization or cytolysis, and part coagulation and fragmentation.

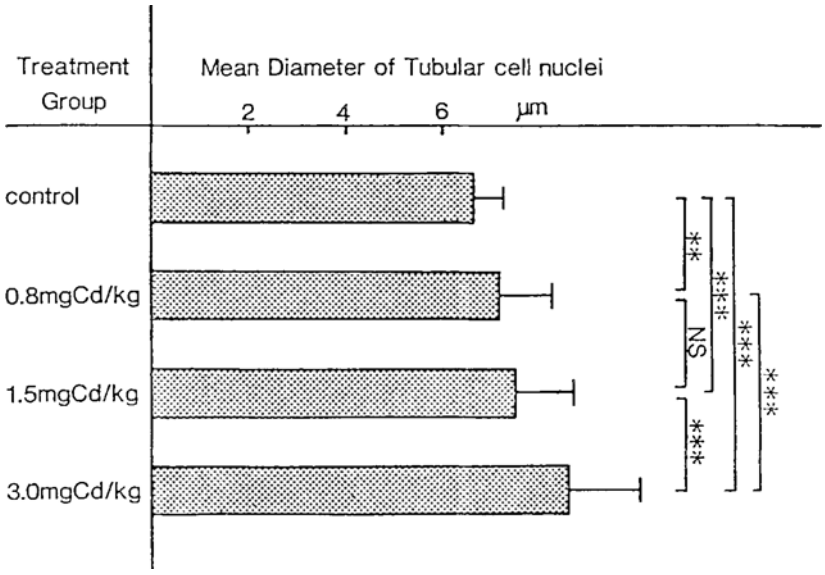

Fig. 1. The change of diameter of tubular cell nuclei. $^{**}p < 0.01$, *** $p < 0.001$.

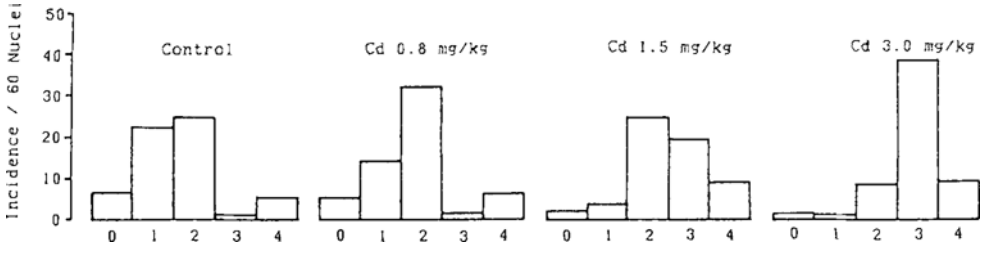

Fig. 2. Grade of nucleolar change in the proximal tubular cells after cadmium exposure (0: no nucleolus in a proximal tubular nucleus; 1: one small nucleolus; 2: one intermediate nucleolus; 3: one large nucleolus; 4: two nucleolei or more). Sixty nuclei in the proximal tubular were graded according to the above criteria, and number of nuclei from five rats were averaged in each group.

A summary of the electron microscopic study in the proximal tubular cells is shown in Table 3. Generally, with the increase in Cd doses, proximal tubular cells developed degeneration and cell death, although their distribution was heterogenous in each nephron and their severity was different from cell to cell in each nephron (Fig. 6).

DISCUSSION

It has been reported that the toxic effect of Cd in the kidney is confined to the cells of proximal tubules, consisting of two distinct histologic features: increase of lysosomes and swelling of mitochondria.

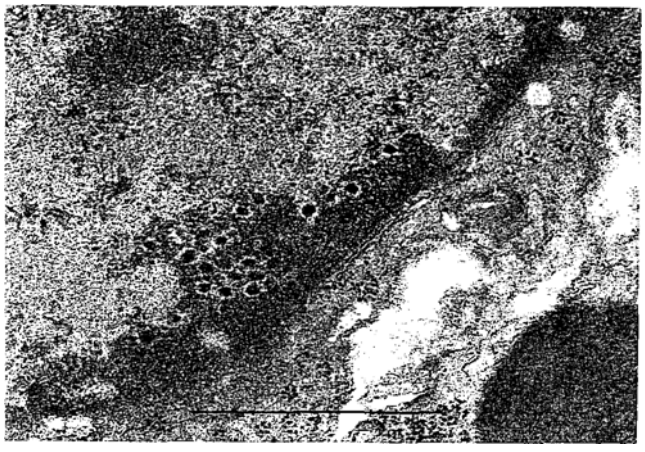

Fig. 3. Proximal tubular nucleus from Cd 0.8 mg/kg group. There are clusters of perichromatin granules at the periphery of the nucleus (1 μm).

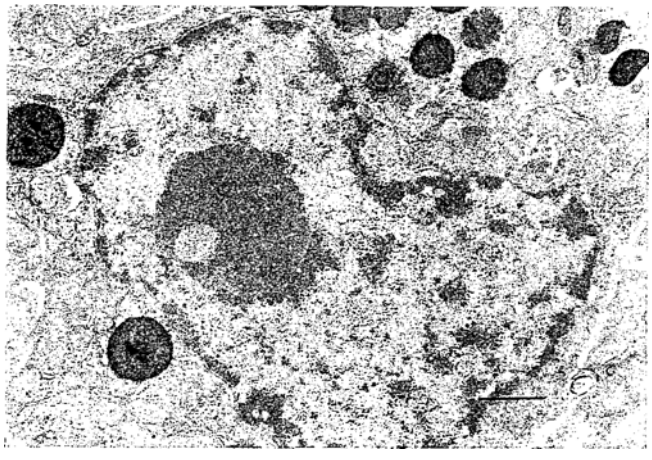

Fig. 4. Proximal tubular nucleus from Cd 3.0 mg/kg group. There is a slight nuclear indentation and a ring-shaped nucleolus with segregation (1 μm).

In addition, some other changes were observed, such as increase of microbodies, focal proliferation of the smooth endoplasmic reticulum, and the appearance of intranuclear inclusions in rats (*10*). Following these observations, Sina and Chin (*11*) reported that exposure of the acellular slime mold Physarum polycephalum to Cd resulted in inhibition of RNA synthesis and in eccentric placement of nucleoli in nuclei, the appearance of multiple nucleolar bodies, ring-shaped nucleoli, and a segregation of fibrillar and granular parts of nucleolus. They suggested that disruption of nucleolar function (i.e., synthesis of RNA) and/or nucleolar structure might be underlying mechanisms of Cd toxicity.

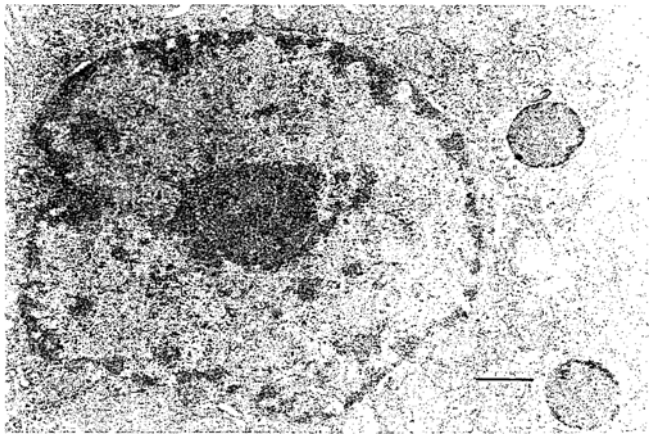

Fig. 5. Proximal tubular nucleus from Cd 0.8 mg/kg group. There is segregated nucleolus with dense granules (1 μm).

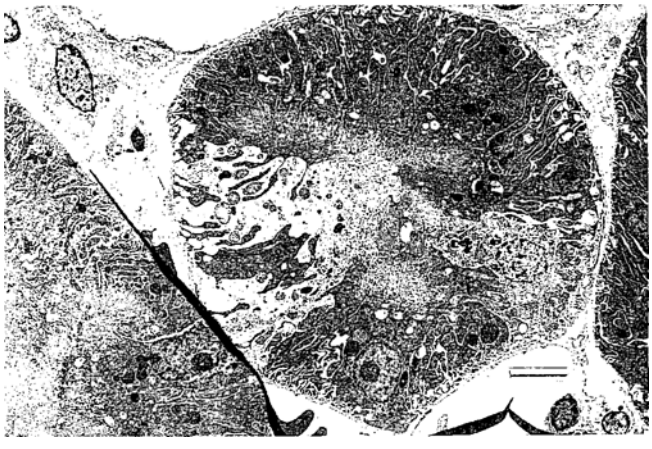

Fig. 6. Proximal tubule from Cd 3.0 mg/kg group. There is a segmented and necrotic cell, and other cells show different stages of degeneration (1:10 μm).

Puvion and Lange (*12*) showed Cd caused chromatin condensation followed by the accumulation of perichromatin granules and an emptying of interchromatin spaces in cultures of liver parenchymal cells. They suggested that these changes also were perhaps owing to inhibition of RNA synthesis. In addition, Ree (*13*) reported nuclear indentation and disruption of nuclear envelope after Cd exposure in an established human epithelial cell line.

In the present study with rats, non-MT-Cd in the 3.0 mg Cd group was decreased, compared with that of the 1.5 mg Cd group. This may

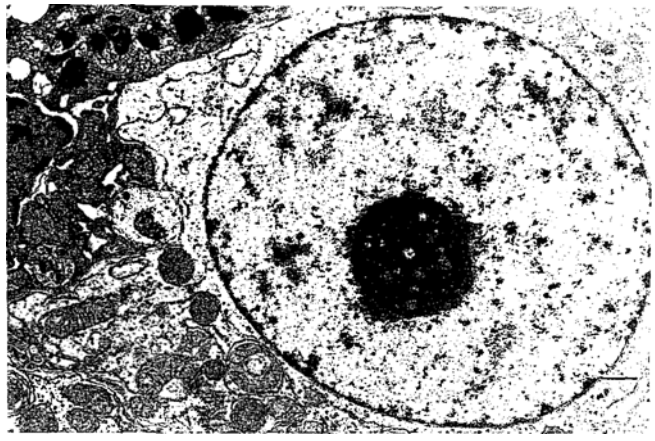

Fig. 7. Proximal tubular nucleus from Cd 3.0 mg/kg group. There is compacted nucleoli with dispersed chromatin (1 μm).

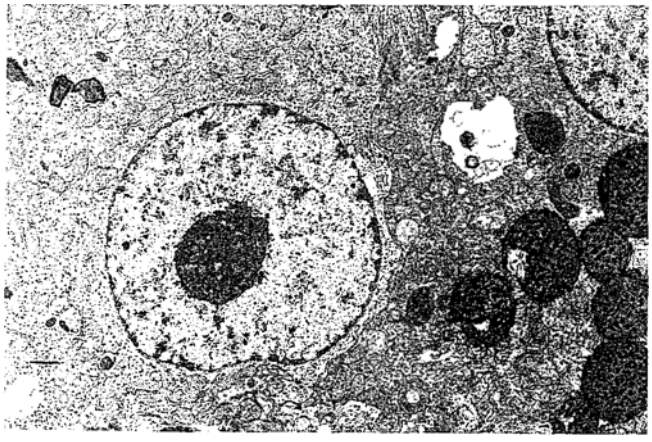

Fig. 8. Proximal tubular cells from Cd 3.0 mg/kg group. Some electron dense granules suggesting lysosomal origin was observed (1 μm).

indicate that non-MT-Cd in the 3.0 mg Cd group had exceeded the critical level for the cells and a significant number of damaged tubular epithelial cells were sequesterated and lost. But less sensitive cells or the cells with ability to produce larger amount of MT remained. Morphologically, although the change was subtle, the renal proximal tubules of Wistar rat given Cd showed chromatin condensation, ring-shaped nucleoli, segregation of nucleolus, and increase in perichromatin granules in a variety of Cd doses. But eccentric placement of nucleoli in nuclei and disruption of nuclear envelope were not observed. Conceivably, their occurence depends on environmental factors or are organ-specific.

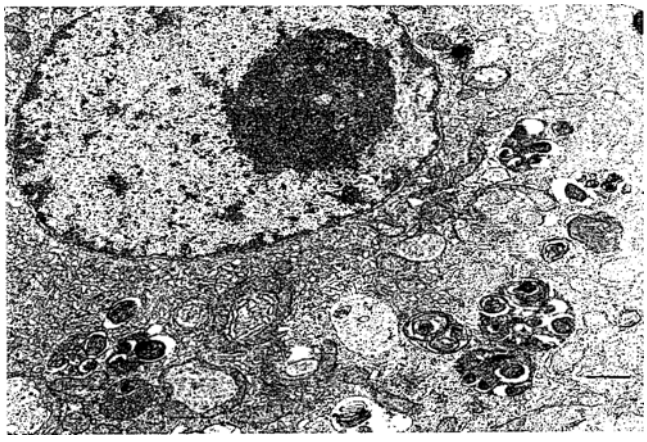

Fig. 9. Proximal tubular cells from Cd 3.0 mg/kg group, containing ring-shaped mitochondria, prominent smooth endosplasmic reticula, and myelin bodies (1 μm).

Table 3
The Summary of Electronmicroscopic Findings

Treatment / findings	Control	Cd 0.8 mg/kg	Cd 1.5 mg/kg	Cd 3 mg/kg
Perichromatin granules	+	+++	+++	++
Dispersed chromatin	–	–	–	+
Nuclear body	–	–	–	+
Ring-shaped nucleoli	+	++	++	+++
Compacted nucleoli	–	–	–	+
Lysosome	+	++	++	+++
Myelin body	–	+	++	+++
Ring-shaped mitochondria	+	+	+	++

There were enlargement and increases in number of nucleolus in the Cd 0.8, 1.5, and 3.0 mg/kg groups with increasing Cd doses. Generally, nucleolar hypertrophy and an increase in numbers of nucleoli are considered to be associated with heightened protein synthesis (*14*). Therefore, this might be as a result of the production of Cd-binding metalloprotein (metallothionein), which is considered to be an accommodative vehicle for protection of the cell (*15,16*).

In our experiments, perichromatin granules increased at low dosage and continued up to the Cd 3.0 mg/kg group. In parallel to these changes, we observed nucleolar hypertrophy, which probably reflects increased syntesis of metallothionein. Thus, inhibition of RNA synthes seems to coexist with an increase in the synthesis of certain other proteins.

However, some of the nucleoli in the Cd 0.8 mg/kg group contained numerous dense granules, and the ring-shaped nucleolus was observed in the Cd 3.0 mg/kg groups. These changes probably reflect regressive changes. The presence of such nucleoli might represent a general phenomenon that reflects a reduced but continuing synthesis of ribosome precursors in the nucleolus (*14*).

Whether these and previously mentioned nucleolar alterations are the result of Cd effects, namely, its ability to interact with and bind to proteins, leading to the formation of Cd–protein complexes in the segregated areas of the nucleolus, or caused by a physical interaction of non-MT-Cd with DNA leading to contraction resulting in nucleolar changes, remains to be established as in the case of tannic acid-induced nucleolar changes in hepatocytes (*17*).

In Cd intoxication, there was the increase of ring-shaped mitochondria in the renal proximal tubules of Cd-treated Wistar rats. It is known that ring-shaped mitochondria may be present in normal and a variety of pathological tissues and states. Such mitochondria are considered to represent a degenerative phenomenon or an adaptive change (*14*). In our experiment, ring-shaped mitochondria remained even in lysed cytoplasm. This change may be secondary to alterations of the cytoplasmic environment.

In the kidneys, the main damage appeared to be the proximal tubules and the extent of the damage was proportional to Cd dose, but there is tubular heterogeneity in these changes. This heterogeneity is perhaps caused by the difference in Cd content in each nephron and cell cycle in each cell.

REFERENCES

1. Copius Peereboom, J. W. and Copius Peereboom-Stegeman, J. H. J., *Toxicol. Environ. Chem. Rev.* **4,** 67–78 (1981).
2. Shenker, B. J., Matarazzo, W. J., Hirsch, R. L., and Gray, I., *Cell. Immunol.* **34,** 19–24 (1977).
3. Stoll, R. E., White, J. F., Miya, T. S., and Bousquet, W. F., *Toxicol. Appl. Pharmacol.*, **37,** 61–74 (1976).
4. Gallagher, K., Matarazzo, W. J., and Gray, I., *Clin. Immunol. Immunopathol.* **13,** 369–377 (1979).
5. Singhal, R. L., Merali, Z., Kacew, S., and Sutherland, D. J. B., *Science,* **183,** 1094–1096 (1974).
6. Krasny, H. C. and Holbrook, D. J., *Mol. Pharmacol.* **13,** 759–765 (1977).
7. Hildebrand, C. E. and Cram, L. S., *Proc. Soc. Exp. Biol. Med.* **161,** 438–443 (1979).
8. Webb, M. in *Metallothionein,* Kagi, J. H. and Nordberg, M., eds., Birkhauser Verlag, Basel/Boston/Stuttgart, pp. 313–320 (1979).
9. Kunifuji, Y., Nakamura, T., and Takasugi, M., *Biol. Trace Element. Res.* **14,** 237–248 (1987).
10. Nishizumi, M., *Arch. Environ. Hlth.* **24,** 215–225 (1972).
11. Sina, J. F. and Chin, B., *Toxicol. Appl. Pharmacol.* **43,** 449–459 (1978).

12. Puvion, E. and Lange, M., *Exp. Cell. Res.* **128,** 47–58 (1980).
13. Ree, K., Rugstad, H. E., and Bakka, A., *Acta Pathol. Microbiol. Immunol. Scand.* (Sect. A.) **90,** 427–435 (1982).
14. Chadially, F. N. *Ultrastructural Pathology of the Cell and Matrix,* 3rd Ed. Butterworths, London/Boston/Singapore/Sydney/Toronto/Wellington, pp. 56–63 (1988).
15. Rugstad, H. E. and Norseth, T., *Nature* (London) **257,** 136,137 (1975).
16. Leber A. P. and Miya, T. S. *Toxicol. Appl. Pharmacol.* **37,** 403–414 (1976).
17. Rao, M. S., Mangino, M. M., Usman, M. I., Subbarao, V., Scarpelli, O. G., Reddy, M. K., and Reddy, J. K., *Cancer Res.* **47,** 1657–1662 (1987).