Br. J. exp. Path. (1983) **64**, 144

EARLY APPEARANCE AND LOCALIZATION OF INTRANUCLEAR INCLUSIONS IN THE SEGMENTS OF RENAL PROXIMAL TUBULES OF RATS FOLLOWING INGESTION OF LEAD

M. MURAKAMI,* R. KAWAMURA,* S. NISHII† AND H. KATSUNUMA‡

*From the *National Institute for Environmental Studies, Yatabe, Ibaraki 305, the †Department of Public Health, Faculty of Medicine, University of Tokyo and the ‡Faculty of Medicine, University of Kyorin, Japan*

Received for publication October 7, 1982

Summary.—Intranuclear inclusions in epithelial cells lining rat renal proximal tubules were detected by electron microscopy as early as 4 days after the addition of lead (5 mg/ml as lead acetate) to the drinking water. At 9 and 12 weeks pathological changes, but very few intranuclear inclusions, were apparent in the epithelial cells lining the third segment of the proximal tubules in the outer stripe of outer zone of the medulla. On the other hand, morphological changes were less in the epithelial cells lining the first and second segments of the proximal tubules in the cortex, which contained many intranuclear inclusions. These findings suggest that lead incorporated into the epithelial cells in the proximal tubules may exist in an inert chemical form as inclusions, especially in the second segment. These bodies, therefore, may protect the cells from the toxic effect of lead.

IT IS WELL KNOWN that many patients with renal disturbance have been found amongst workers exposed to lead for a long time (Lilis, 1968) and in adults who had ingested a lot of flakes of lead paint in their childhood (Henderson, 1958).

It is not evident yet why and how the lead induces renal disturbance. It is reasonable to assume, however, that in any lead-poisoning cases, whether due to occupational or accidental exposure in infancy, large amounts of lead have been incorporated into various body-tissues.

Intranuclear inclusions have been found in the renal epithelial cells of lead-poisoned children (Blackman, 1936) and chronically lead-administered animals (Tönz, 1957). It has been confirmed by chemical analysis of kidney homogenate (Goyer *et al.*, 1970*a*), autoradiography (Dallenbach, 1965) and electron probe X-ray microanalysis (Carroll, Spinelli and Goyer, 1970; Anders *et al.*, 1982) that these intranuclear inclusions contain lead. The existence of this type of lead-induced inclusion has been reported in various kinds of cells such as hepatic cells (Blackman, 1936), osteocrast cells (Hsu *et al.*, 1973), renal epithelial cells in tissue culture (Walton and Buckly, 1977) and even cells of plants (Skaar, Ophus and Gullvag, 1973).

These facts suggest that the appearance of intranuclear inclusions is not a phenomenon specific to renal proximal tubular epithelium and may be common to those cells that accumulate large amounts of lead.

Although intranuclear inclusions had been thought to be a characteristic sign of chronic lead poisoning in man (Angevine *et al.*, 1962), it has been reported that these inclusions appear at a very early time in cultured kidney cells exposed to lead (McLachlin. Goyer and Cherian, 1980) and

Correspondence to: Dr M. Murakami, National Institute for Environmental Studies, Yatabe, Ibaraki 305, Japan.

in the kidney proximal cells of the rat following intracardiac injection of lead (Choie, Richter and Young, 1975).

This study was designed to determine (i) the time at which intranuclear inclusions appear in the rat kidney following the addition of lead to the drinking water, (ii) whether intranuclear inclusions occur more frequently in the epithelial cells of one segment of the nephron than in those of others and (iii) if any relationship exists between the cellular pathological changes and the presence of intranuclear inclusions.

MATERIALS AND METHODS

Sixty male rats, Donryu strain, 10 weeks old, weighing 360 ± 59 g were divided into 12 groups of 5 rats. Seven groups were given tap water containing 5 mg of lead/ml (as lead acetate) for the following periods: (1) 4 days, (2) 1 week, (3) 2 weeks, (4) 3 weeks, (5) 6 weeks, (6) 9 weeks, (7) 12 weeks. Four groups (8–11) were given tap water containing 0·5 mg of lead/ml for (8) 3 weeks, (9) 6 weeks, (10) 9 weeks, (11) 12 weeks. The control group was given tap water for 12 weeks. All animals were fed standard laboratory chow *ad libitum*. Body weights and blood haemoglobin were measured every 3 weeks.

After termination of treatment, the animals were decapitated. Samples of blood were taken immediately and the kidneys were excised. The right kidneys and 10 ml of blood were ashed in the plasma ashing apparatus and they were dissolved into 10 ml nitric acid. Lead was determined by atomic absorption spectrophotometry.

Blocks of the left kidneys were fixed with 4% paraformaldehyde, 2·5% glutaraldehyde buffered with 0·1M sodium phosphate for 1 h and then were postfixed with 1% osmic acid buffered with 0·1M sodium phosphate for 2 h. After dehydration with graded ethanols and clearing with propylen oxide, the blocks were embedded in Epon mixture. 1 μm thick sections were stained with toluidene blue for light microscopical observations and ultrathin sections stained, with lead and uranium, were examined in a Hitachi HS-8 type electronmicroscope.

In order to ascertain the incidence of intranuclear inclusions, 200 nuclei were observed in the epithelial cells lining the proximal tubules in the cortex by light microscopy and under high magnification (up to × 20,000) by electron microscopy.

In order to identify the elements in the concretions of the epithelial cells lining proximal tubules, thin renal cortical sections from rats, exposed to 5 mg lead/ml drinking water for 12 weeks were placed on nylon mesh and analysed by non-dispersive X-ray microanalysis.

RESULTS

Average water consumption by the low-dose (0·5 mg lead/ml) group was slightly less (450 ml/week/animal) than that of the control group (480 ml/week/animal), but apparently greater than that (280 ml/week/animal) of the high-dose group (5 mg lead/ml). Estimated average intake of lead by the animals of the low- and high-dose groups, therefore, were 32 mg/day and 200 mg/day, respectively.

Body weight changes up to 12 weeks were shown in Table I. Weight gain in the high-dose animals was suppressed throughout the experimental period, but was unaffected in the low-dose group even at 12 weeks.

Blood haemoglobin concentration was decreased, relative to the control, after 3 weeks exposure in the high-dose group but was unaffected at any time in the low-dose group (Table II).

The lead concentration of kidneys and blood increased in proportion to the administered dose of lead as shown in Table III.

At any time in the high-dose group and at longer than the 6 weeks in the low-dose groups, the average value of the renal lead concentration was more than 10 μg/g.

Average blood lead concentrations of treated animals were more than 1·0 μg/g (100 μg/dl) except in those of the low-dose group at 3 weeks.

The incidence of intranuclear inclusions in each group is shown in Table IV. Intranuclear inclusions were detected by electron microscopy in very few epithelial cells of proximal tubules as early as 4 days after the addition of lead to the drinking water.

In the high-dose group, exposure times longer than 6 weeks resulted in an increase in kidney weight (Table V), possibly indicative of renal oedema.

TABLE I.—*Body weight at different times after administration of lead drinking water (g)*

	Duration (weeks) N	0	3	6	9	12
Control	4	362 ± 27	399 ± 31	430 ± 35	433 ± 46	440 ± 30
0·5 mg/ml	5	367 ± 15	401 ± 26	437 ± 24	451 ± 35	464 ± 35
5 mg/ml	5	360 ± 14	359 ± 21	388 ± 23	396 ± 19	411 ± 20

N = number of animals.

TABLE II.—*Haemoglobin at different times after administration of lead drinking water (g/dl blood)*

	Duration (weeks) N	0	3	6	9	12
Control	5	13·4 ± 0·5	13·1 ± 0·2	13·5 ± 0·1	12·9 ± 0·5	13·5 ± 0·5
0·5 mg/ml	5	13·2 ± 0·6	13·5 ± 0·4	13·1 ± 0·7	12·7 ± 0·7	13·0 ± 0·1
5 mg/ml	5	13·5 ± 0·1	11·5 ± 0·1**	12·1 ± 0·1**	12·0 ± 0·1*	11·8 ± 0·1*

Significantly different from the control ($P < 0·05$*, $P < 0·01$**) by the Student's t test.
N = number of animals.

Light microscopical findings

In the high dose group at 12 weeks, pathological changes were prominent in the outer stripe of the outer zone of the medulla. The third (S3) segments of the proximal tubules had lost their characteristic appearance and appeared very irregularly deformed with a concomitant increase and swelling of the interstitial tissue. The epithelial cells lining S3 segment of proximal tubules were variously tall and contained many nuclei. Their cytoplasm stained irregularly and some cells were without brush borders. It seems, therefore, that at this time some degenerated cells and regenerated cells exist in the S3 segment of proximal tubules in the outer stripe (Fig. 1).

Less morphological change was observed in the cortex, compared to the outer stripe. Epithelial cells lining the second segment (S2) of the proximal tubules, which contained numerous lysosomes and stained clearly with toluidine blue, showed many enlarged inclusions. Intranuclear inclusions were found to be spherical or ovoid, to stain positively in the toluidine blue reaction (Richter, Kress and Cornwall, 1968) and, because of their more intense staining with toluidine blue and fibrilar structure, to be differentiated easily from nucleoli and aggregated chromatin. These inclusion bodies consisted of central and peripheral parts, the former of which stained more intensively than the latter.

The epithelial cells lining the first (S1) segments of the proximal tubule which contained fewer lysosomes than those of the S2 segment, also contained a smaller number of intranuclear inclusions. The morphological changes in these cells were as moderate as in those of the epithelial cells lining S2 of proximal tubules (Fig. 2).

The damage to epithelial cells lining the different segments of the proximal tubules thus regressed in the following order: S3 in the outer stripe of outer zone of the medulla. S2 and S1 in the cortex. Some concretions, which were coloured bright brown, were observed in the lumen and the apical cytoplasm of proximal tubules, especially in the S2 segments (Fig. 3). These had a spherical or ovoid shape and their diameters reached up to 10 μm.

At 9 weeks in the high-dose group, the interstitial tissue in the outer stripe appeared to be slightly increased. The S3 segments of proximal tubules in the outer stripe were irregularly deformed and the epithelial cells lining these segments, which stained very variably with toluidine blue, were increased and some of them were degenerated (Fig. 4). Numerous clearly stained intranuclear inclusions were observed especially in the epithelial

TABLE III.—*Lead concentration of blood and kidney at different times after administration of lead drinking water (μg/g wet wt tissue)*

Blood							
Duration	4 days	1 week	2 weeks	3 weeks	6 weeks	9 weeks	12 weeks
Control							0·07 ± 0·03 (N = 5)
0·5 mg/ml				0·78 ± 0·58 (N = 5)	1·63 ± 0·70 (N = 4)	2·08 ± 0·87 (N = 5)	2·70 ± 0·83 (N = 5)
5 mg/ml	1·04 ± 0·50 (N = 5)	1·00 ± 0·18 (N = 5)	1·05 ± 0·22 (N = 5)	2·00 ± 1·21 (N = 5)	4·60 ± 1·20** (N = 5)	3·56 ± 1·19 (N = 5)	8·68 ± 5·02 (N = 4)
Kidney							
Duration	4 days	1 week	2 weeks	3 weeks	6 weeks	9 weeks	12 weeks
Control							0·13 ± 0·19 (N = 5)
0·5 mg/ml				4·16 ± 1·38 (N = 5)	11·58 ± 2·16 (N = 4)	———	14·98 ± 7·27 (N = 5)
5 mg/ml	12·54 ± 4·20 (N = 5)	19·72 ± 2·80 (N = 5)	10·97 ± 2·30 (N = 5)	13·58 ± 3·26** (N = 4)	37·30 ± 17·10 (N = 4)	———	148·7 ± 41·3** (N = 4)

Significantly different from the low-dose group ($P < 0·05$*, $P < 0·01$**) by Student's t test.
N = number of animals.

TABLE IV.—*Incidence of intranuclear inclusion bodies in the renal proximal tubular epithelial cells of the rat at different times after exposure to lead in the drinking water*

Duration	4 days	1 week	2 weeks	3 weeks	6 weeks	9 weeks	12 weeks
0·5 mg/ml				0/5	1/4	5/5	5/5
5 mg/ml	3/5	4/5	5/5	5/5	5/5	5/5	4/4

TABLE V.—*Kidneys weight at different times after administration of lead drinking water* (*g*)

Duration	3 weeks	6 weeks	9 weeks	12 weeks
Control				1·54±0·38 (N=5)
0·5 mg/ml	1·68±0·15 (N=5)	1·83±0·14 (N=4)	1·62±0·11 (N=5)	1·63±0·08 (N=5)
5 mg/ml	1·57±0·19 (N=4)	2·01±0·13 (N=4)	2·00±0·22** (N=5)	1·78±0·22 (N=4)

Significantly different from the low-dose group ($P<0·05$*, $P<0·01$**) by Student's *t*-test.
N = number of animals.

cells of the S2 segments of the proximal tubules (Fig. 5). In these cases, bright brown concretions were not found anywhere in the kidney.

At 6 weeks in the high-dose group, no increase of interstitial tissue was observed but the epithelial cells of the S3 segments were slightly deformed in comparison with the control. A few intranuclear inclusions were found in the epithelial cells lining convoluted proximal tubules.

No pathological changes were observed in either the high-dose group before 3 weeks or the low-dose group during the first 9 weeks of lead exposure. Although filamentous intranuclear inclusions were detected in the kidneys of some of these animals by electron microscopy (see below), these were too small to be resolved by light microscopy.

Electron microscopical findings

At 4 days and 1 week in the high-dose group and at 6 weeks in the low-dose group, morphological changes in proximal tubular epithelial cells were limited to the appearances of a few intranuclear inclusions. These inclusions were variously shaped nets of filament of 50–200 Å in width (Fig. 6).

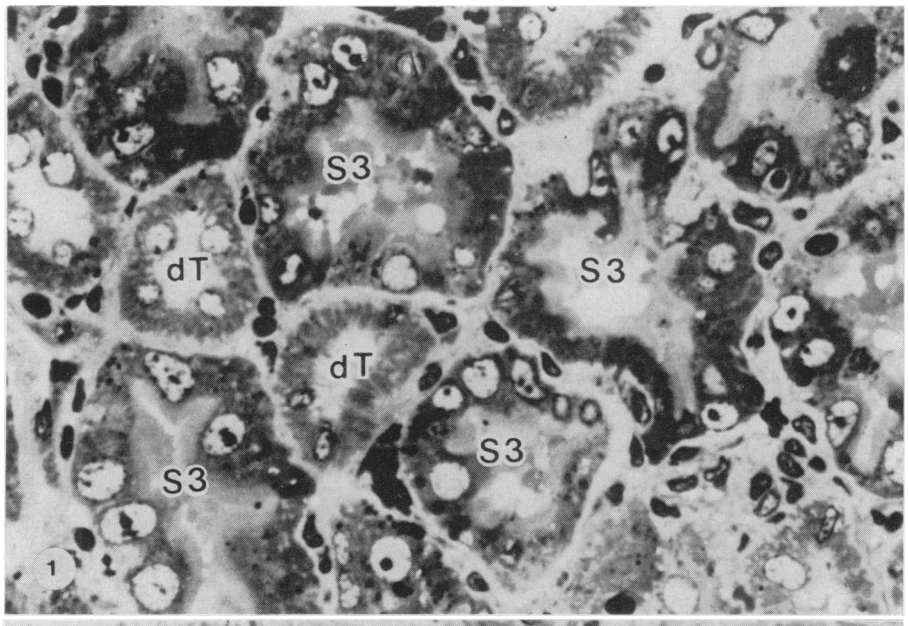
At 2 and 3 weeks in the high-dose group and at 9 weeks in the low-dose group, intranuclear inclusions increased in number and grew larger (*i.e.* from 0·5–2·0 μm). The fine structure of these inclusions consisted of a peripheral part and a central part. The former showed filamentous structures which were radially oriented. The width of these filaments ranged from 50–200 Å. The central part was denser than the peripheral part and appeared as a composite of filaments and dense amorphous substance (Fig. 7).

At 6 and 9 weeks in the high-dose group, many mature intranuclear inclusions were present. These were much larger in size than those at earlier times and the peripheral and central parts were differentiated clearly (Fig. 8).

At 12 weeks in the high-dose group, many epithelial cells contained a number of mature intranuclear inclusions, which ranged in size from 2–4 μm in the larger nuclei. In addition the concretions, which appeared to be brown coloured by light microscopy, were observed as very electron dense material. And X-ray microanalysis showed that these concretions contained lead and calcium (Figs 3, 9 and 10).

DISCUSSION

It has been reported that at least 4 weeks is necessary to form intranuclear inclusions in renal proximal tubular cells, when animals are given drinking water



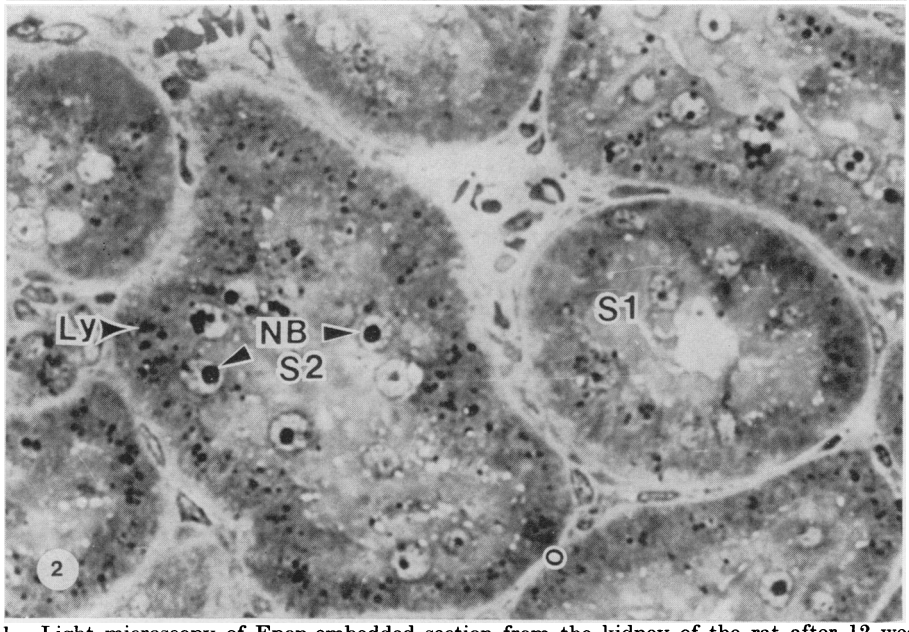


FIG. 1.—Light microscopy of Epon-embedded section from the kidney of the rat after 12 weeks exposure to the high dose of lead. The S3 segment of proximal tubules (S3) have lost their characteristic appearance, and are very irregularly deformed with a concomitant increase and swelling of the interstitial tissue. The epithelial cells are variously tall, stain very variously in their cytoplasm, contain many nuclei and are with or without brush borders. dT (distal tubule). Toluidine blue (× 1,200).

FIG. 2.—Light microscopy of Epon-embedded section from the kidney of the rat after 12 weeks exposure to the high dose of lead. The epithelial cells lining S2 segment of proximal tubules (S2) in which numerous lysosomes (Ly arrow) are apparent, stain clearly with toluidine blue and contain many enlarged inclusions (NB arrow). Intranuclear inclusions are found to be spherical or ovoid and to stain positively in the toluidine blue reaction. The epithelial cells lining S1 segment of proximal tubules (S1), which contain fewer lysosomes than those of S2 segment, also contain fewer intranuclear inclusions than S2 segment (× 1,200).

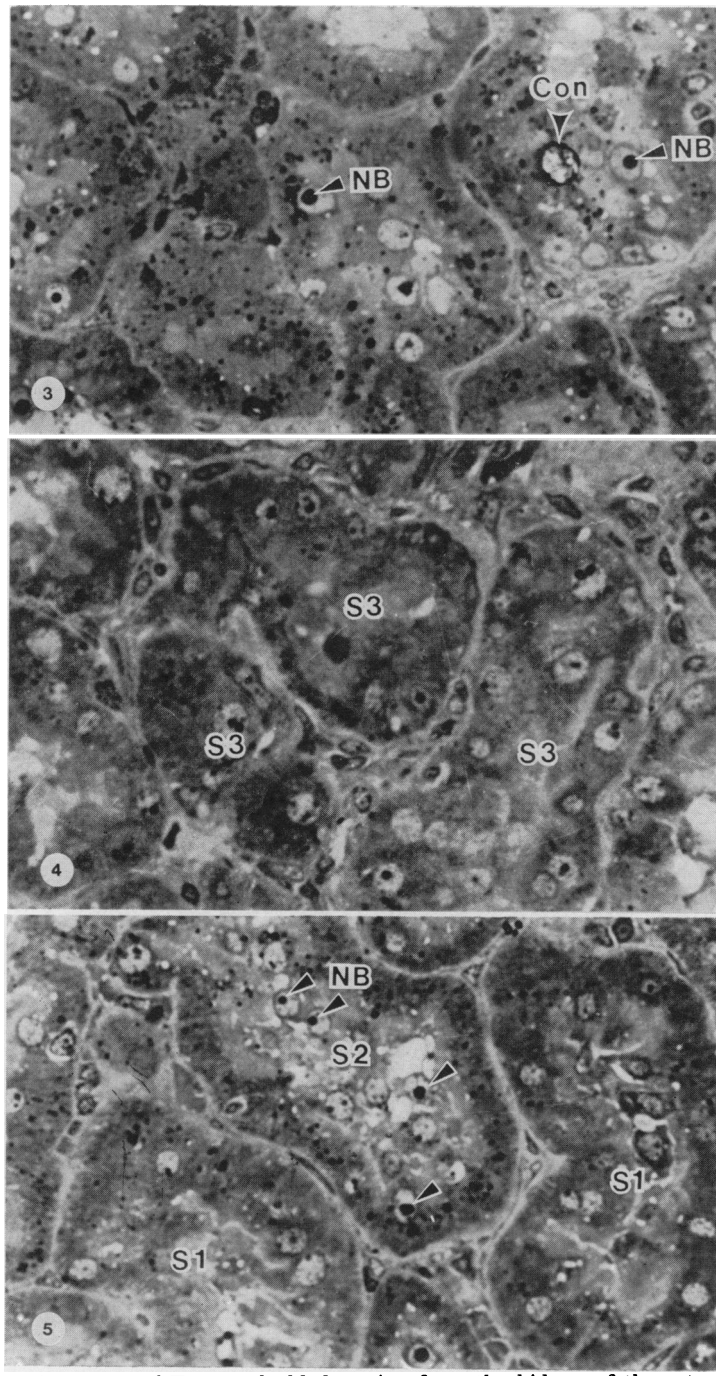
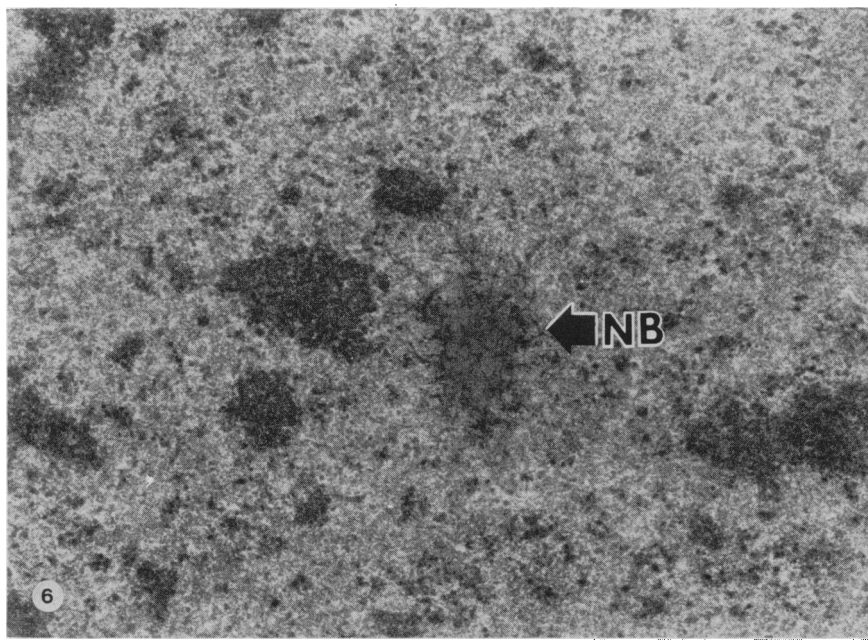


FIG. 3.—Light microscopy of Epon-embedded section from the kidney of the rat after 12 weeks exposure to the high dose of lead. A bright brown concretion (Con) is apparent (arrow) in the apical site of the epithelial cell lining the convoluted proximal tubule. Toluidine blue (×960).

FIG. 4.—Light microscopy of Epon-embedded section from the kidney of the rat after 9 weeks exposure to the high dose of lead. Interstitial tissue in the outer stripe is slightly increased. S3 segment of proximal tubules (S3) in the outer stripe are irregularly deformed and the epithelial cells lining these segments are increased. The cells stain very variously with toluidine blue and some of them are degenerated (×960).

FIG. 5.—Light microscopy of Epon-embedded section from the kidney of the rat after 9 weeks exposure to the high dose of lead. Numerous clearly stained intranuclear inclusions (NB) are observed especially in the epithelial cells of S2 segment of proximal tubules (S2). Toluidine blue (×960).



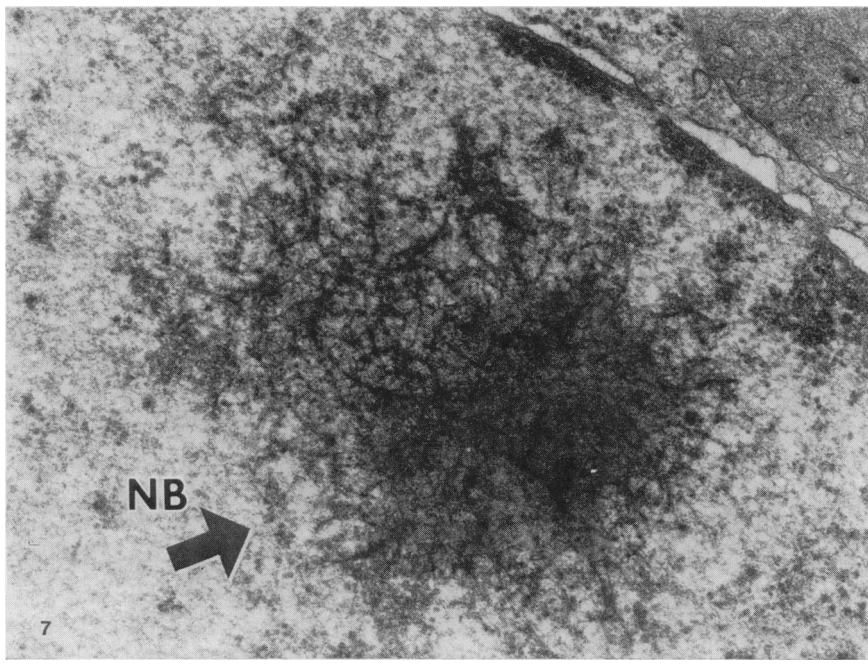


FIG. 6.—Electron microscopy of the proximal tubular epithelial cell after 4 days exposure to the high dose of lead. No morphological changes are observed in the epithelial cell except for appearance of very few intranuclear inclusions (NB). These intranuclear inclusions are variously shaped nets of filaments of 50–200 Å in width (× 24,000).

FIG. 7.—Electron microscopy of the proximal tubular epithelial cell after 2 weeks exposure to the high dose of lead. The fine structure of intranuclear inclusion (NB) consists of the peripheral part and the central part. The latter part is denser than the former and appears as a composite of filaments and dense amorphous substance. The filaments vary in width from 50–200 Å and are radically oriented (× 32,250).

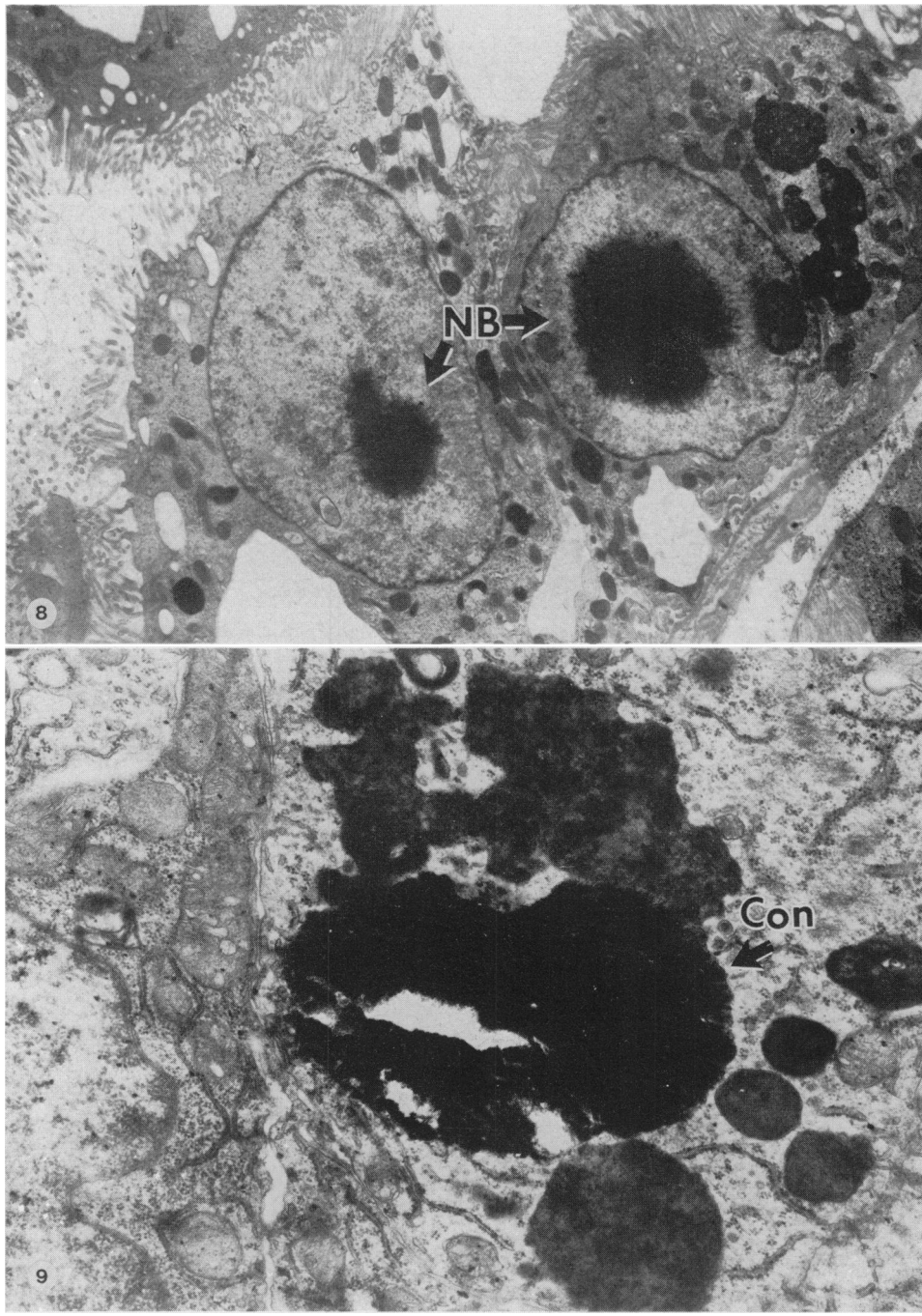


FIG. 8.—Electron microscopy of the proximal tubular epithelial cells after 9 weeks exposure to the high dose of lead. Many mature intranuclear inclusions (NB). These are larger in size than at earlier times and the peripheral and central parts are clearly differentiated (× 11,250).

FIG. 9.—Electron microscopy of the proximal tubular epithelial cell after 12 weeks exposure to the high dose of lead. A concretion (Con), which appears to be bright brown coloured by light microscopy (Fig. 3), is observed as very electron dense material (arrow) (× 23,250).

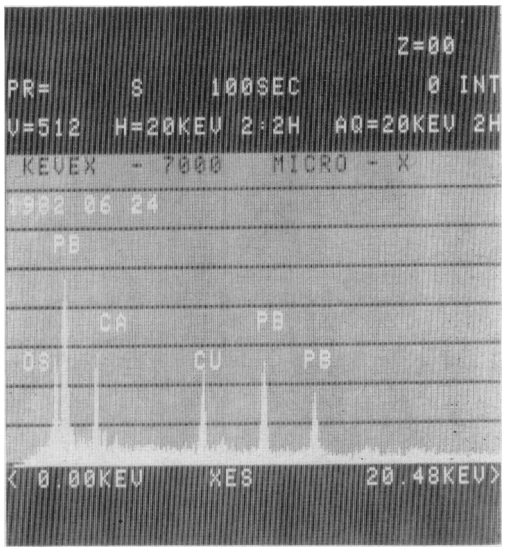


FIG. 10.—X-ray microanalysis of the concretion shown in Fig. 9. This contains Pb and Ca, although Os and Cu come from the fixative and the mesh respectively.

containing 0·1% lead acetate (Beaver, 1961). In contrast, intranuclear inclusions have been observed in mouse renal cortex at 6 h after intracardiac injection of lead (Choie *et al.*, 1975). Also in primary rat kidney epithelial cell cultures, inclusions were detected in the cytoplasm within 4 h and in the nucleus at 24 h of exposure to a medium containing lead nitrate (McLachlin *et al.*, 1980). Furthermore, Walton and Buckly (1977) observed inclusions in the nucleus at 1 h after exposure of kidney tubule cell cultures to lead in sucrose solution. In this study, intranuclear inclusions were found also in the proximal tubular epithelial cells of rats which were given drinking water containing 5 mg of lead/ml for only 4 days. Irrespective of the dose level and duration of exposure, it seems that intranuclear inclusions appear in the renal proximal tubular cells once the kidney concentration of lead exceeds 10 μg/g tissue.

This concentration value is the same as for renal lead concentration, when intranuclear inclusions appeared in the kidneys of rats which were fed a conventional diet containing 1% lead acetate for 10 weeks (Goyer *et al.*, 1970*b*), although the renal lead concentration required to initiate intranuclear inclusions in the animal exposed to lead through the other way remained unknown (Choie *et al.*, 1975; Richter *et al.*, 1968; Choie and Richter, 1972; Müller and Ramin, 1963) except for oral treatment.

At 4 days, the earliest time at which intranuclear inclusions were observed electronmicroscopically in the high-dose group of the present experiments, the effects of lead on body weight and haemoglobin concentration would be minimal. At this time no morphological changes, other than appearance of intranuclear inclusions, are detectable electronmicroscopically. The appearance of intranuclear inclusions, therefore, would seem to be a sensitive indicator of lead exposure. Goyer also reported these bodies to be the most sensitive indicator among the renal pathological changes, whether morphological or functional (Goyer *et al.*, 1970*b*).

At early times, intranuclear inclusions are very small round filamentous structures and sometimes only a rough filamentous net (Richter *et al.*, 1968) which may be a precursor or inclusion composed of a type of protein (Choie *et al.*, 1975). They are clearly differentiated from chromatin granules, by their filamentous structure, which is revealed by electron microscopy.

As many investigators have reported previously, as exposure to lead continues, intranuclear inclusions enlarge and, because they are acid-fast on the light microscopical level, they stain positively with the toluidine blue reaction (Richter *et al.*, 1968). The number of proximal tubular cells containing enlarged intranuclear inclusions also increases.

In this study, the intranuclear inclusions have been shown to be concentrated in the epithelial cells lining the second segment of proximal tubules. Lead is known to accumulate to a greater extent in the proximal tubules than in other portions of the nephron such as glomeruli,

distal tubuli and collecting tubes (Dallenbach, 1965).

The biological signifiance of the intranuclear inclusions in the lead nephropathy is not yet clear. However, many investigators have postulated that they serve to protect cells from the toxic effects of lead on cell metabolism (Goyer and Rhyne, 1973). The present study shows clearly that pathological changes are severe in S3 of proximal tubules containing few intranuclear inclusions, moderate in S1 with a few intranuclear inclusions and in S2 with numerous intranuclear inclusions. These findings, which suggest that lead incorporated into the epithelial cells in the proximal tubules may exist in an inert chemical form as inclusions, especially in the second segment, provide further evidence for the protective function of these bodies.

The same type of concretions previously reported by Murakami (Murakami, 1971*a*, *b*), have been observed in the renal cortex containing 149 μg/g lead (*i.e.* at 12 weeks exposure in the high-dose animals). These concretions are so hard that they damage glass knives and are composed of lead and calcium. They have been reported previously in the rat (Tönz, 1957; Murakami, 1971*a*; Eger, 1937) and rabbit (Jores, 1902) after chronic exposure to lead.

These findings suggest that the formation of concretions occurs when the kidney accumulates large amounts of lead, as a result of either prolonged or high exposure. There are no reports that workers exposed to large amounts of lead suffered from stones in urological organs, although some chronically poisoned patients sometimes complained of abdominal colic. It is possible, however, that concretions occur in humans also, but more intensive clinical and epidemiological study would be necessary to establish this.

The authors are grateful to Dr M. Webb of the Toxicology Unit, British MRC Laboratories, for his comments and suggestions and to Miss K. Miyasaka for her help with X-ray microanalysis.

REFERENCES

ANDERS, E., DIETZ, D. D., BAGNELL, C. R. JR., GAYNOR, J., KRIGMAN, M. R., ROSS, D. W., LEANDER, J. D. & MUSHAK, P. (1982) Morphological, Pharmacokinetic, and Hematological Studies of Lead-Exposed Pigeons. *Environ. Research*, **28**, 344.

ANGEVINE, J. M., KAPPAS, A., DEGOWIN, R. L. & SPARGO, B. H. (1962) Renal Tubular Nuclear Inclusions of Lead Poisoning. *Arch. Path.*, **73**, 486.

BEAVER, D. L. (1961) The Ultrastructure of the Kidney in Lead Intoxication with Particular Reference to Intranuclear Inclusions. *Am J. Pathol.*, **139**, 195.

BLACKMAN, S. S. (1936) Intranuclear Inclusion Bodies in the Kidney and Liver Caused by Lead Poisoning. *Bull. Johns Hopkins Hosp.*, **58**, 384.

CARROLL, K. G., SPINELLI, F. E. & GOYER, R. A. (1970) Electron Probe Microanalyzer Localization of Lead in Kidney Tissue of Poisoned Rats. *Nature*, **227**, 1056.

CHOIE, D. D. & RICHTER, G. W. (1972) Lead Poisoning: Rapid Formation of Intranuclear Inclusions. *Science*, **177**, 1194.

CHOIE, D. D., RICHTER, G. W. & YOUNG, L. B. (1975) Biogenesis of Intranuclear Lead-Protein Inclusions in Mouse Kidney. *Beitr. Path. Bd.*, **155**, 197.

DALLENBACH, H. E. (1965) Die Aufname von Radioactivem Blei 210 Durch die Tublusepitheliender Niere. *Verh. Dtseh. Ges. Pathol.* **49**, 179.

EGER, W. (1937) Experimentelle Bleishrumpfnieren. *Virchows Arch.*, **299**, 654.

GOYER, R. A., MAY, P., CATES, M. & KRIGMAN, M. R. (1970*a*) Lead and Protein Content of Isolated Intranuclear Inclusion Bodies from Kidneys of Lead Poisoned Rats. *Lab. Invest.*, **22**, 245.

GOYER, R. A., LEONARD, D. L., MOORE, J. F., RHYNE, B. & KRIGMAN, M. R. (1970*b*) Lead Dosage and the Role of the Intranuclear Inclusion Body. *Arch. Environ. Health*, **20**, 705.

GOYER, R. A. & RHYNE, B. C. (1973) In *International Review of Experimental Pathology*, Vol. 12. (Eds Richter and Epstein), New York: Academic Press, p. 1.

HENDERSON, D. A. (1958) The Aetiology of Chronic Nephritis in Queensland. *Med. J. Aust.*, **1**, 377.

HSU, F. S., KROOK, L., SHIVELY, J. N. & DUNCAN, J. R. (1973) Lead Inclusion Bodies in Osteoclasts. *Science*, **181**, 447.

JORES, L. (1902) Ueber die Pathologie anatomie der Chronischen Bleivergiftung des Kaninchens. *Beit. Path. Anat. allge. Path.*, **31**, 183.

LILIS, R. (1968) Nephropathy in Chronic Lead Poisoning. *Br. J. Industr. Med.*, **25**, 196.

MCLACHLIN, J. R., GOYER, R. A. & CHERIAN, M. G. (1980) Formation of Lead-Induced Inclusion Bodies in Primary Rat Kidney Epitherial Cell Cultures: Effect of Actinomhcin D and Cycloheximide. *Toxicol. Appl. Pharmacol.* **56**, 418.

MÜLLER, H. A. & RAMIN, D. V. (1963) Morphologie und Morphogenese der Durch Schwermetalle (Pb, Bi) Hervorgerufenen Kereinschlüsse in den Hauptstückepithelien der Rattenniere. *Beitr. Path. Anat.*, **128**, 445.

MURAKAMI, M. (1971*a*) Deposition of Lead in the Kidney of Rats with Lead Poisoning. *Japan. J. Ind. Health*, **13**, 433.

MURAKAMI, M. (1971*b*) Identification of Elements of "Concretion" in the Kidneys of Rats with Experimental Lead Poisoning. *Ind. Health*, **9**, 194.

RICHTER, G. W., KRESS, Y. & CORNWALL, C. C. (1968) Another Look at Lead Inclusion Bodies. *Am. J. Pathol.*, **53**, 189.

SKAAR, H., OPHUS, E. & GULLVAG, B. M. (1973) Lead Accumulation within Nuclei of Moss Leaf Cells. *Nature*, **241**, 215.

TÖNZ, O. (1957) Nierenveränderungen bei Experimenteller Chronischer Bleivergiftung (Ratten) *Z. Exper. Med.*, **128**, 361.

WALTON, J. & BUCKLY, J. K. (1977) The Lead-Poisoned Cell: A Fine Structural Study Using Cultured Kidney Cells. *Exp. Mol. Path.* **27**, 167.