Review Article


Nephrotoxicity of cadmium & lead

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Cadmium and lead are divalent cations with a propensity to settle in the proximal tubule of the nephron, leading to nephrotoxicity. The pathophysiological results, however, tend to diverge. Cadmium in sufficient cumulative dosage leads to the production of the Fanconi syndrome, a generalized proximal tubular reabsorptive defect thought to be related to inhibition of both ATP production and Na-K-ATPase activity. On the other hand, lead accumulation in the proximal tubule leads to hyperuricaemia and gout, presumably by inhibiting uric acid secretion, and diminished glomerular filtration rate (GFR). Fanconi syndrome is seen unusually only in children and experimental animals. Cadmium nephrotoxicity is heralded by increased excretion of β2-microglobulin, retinol binding protein and α1-microglobulin, indicative of decreased proximal tubule function. Beta2-microglobulinuria is not found in lead nephropathy. In lead nephropathy albuminuria is absent or minimal whereas in cadmium nephropathy albuminuria is variable. From the standpoint of pathology, both entities are characterized by tubulointerstitial disease and fibrosis, but only early lead nephropathy is characterized by the presence of proximal tubule nuclear inclusion bodies, due to the combination of lead with a lead binding-protein.

Key words Cadmium - Fanconi syndrome - glomerular filtration rate - gout - kidney - lead - nephrotoxicity - proteinuria

Introduction

Cadmium and lead are two of the most prevalent as well as two of the most nephrotoxic metals known to man. The focus of this review is to compare and contrast the clinico-pathological presentations and the pathogenesis of the nephrotoxicity of each of these metals.

CADMIUM

Cadmium exposure sources

Nephrotoxicity caused by cadmium has been described in settings of industrial exposure and environmental pollution. Cadmium, a metal ordinarily obtained as a by-product of zinc refining, is used industrially in plating of steel, pigments, plastics, alloys, and nickel-cadmium batteries, and in nuclear and electronic engineering. Because the biologic half-life of cadmium is long (more than 30 yr), prolonged low-level exposure leads to excessive accumulation in certain tissues, especially the kidney. Studies of cadmium toxicity have been performed in the accumulator battery industry, the copper-cadmium alloy industry, and cadmium pigment factories. Chronic poisoning typically was found to have occurred after several years of exposure and was characterized clinically by variable features of nasorespiratory involvement, such as emphysema, rhinitis, alteration...
of nasal mucosa, and anosmia, and by renal tubular dysfunction. Yellow tooth discolouration, mild anaemia, and disturbances in calcium metabolism, osteomalacia, and renal toxicity also were observed occasionally. Environmental cadmium exposure occurs in residents living in proximity to industrial pollution and also in heavy smokers, as tobacco smoke yields high cadmium concentrations.

Clinically detectable proteinuria (i.e., by the sulphosalicylic or heat and acetic acid tests) is not seen until at least nine and more commonly 25 years of exposure. Initially, the proteinuria was thought to be exclusively of tubular origin (i.e., low molecular weight). Indeed, when sensitive tests for low molecular weight proteinuria were used (electrophoresis, immunoassays for β2-microglobulin, α1-microglobulin, and retinol binding protein, or enzymatic tests for lysozyme and ribonuclease) these tests were positive in workers with lesser degrees of exposure and in whom clinical proteinuria was absent. Later it was found in both workers and experimental animals exposed to cadmium that the proteinuria is a mixed type, with increased excretion of both high- and low-molecular weight proteins, indicative of both glomerular protein loss and decreased tubular reabsorption.

Thresholds

The threshold at which tubular proteinuria appears has been defined in various ways. Initially the threshold was set at a urinary cadmium value of 10 μg/g creatinine, reflecting increased kidney levels of cadmium. The threshold for kidney cortex cadmium was similarly set at 200 μg/g wet weight. Over the subsequent years, the threshold for urinary cadmium has been gradually reduced as more sensitive tests have been developed. Alpha 1-microglobulin has commonly been substituted for β2-microglobulin as a marker for tubular proteinuria as the latter is sensitive to pH changes whereas the former is not. Another low molecular weight protein, metallothionein, which is produced in response to cadmium exposure, is also excreted in increased quantities when the body burden of cadmium exceeds a threshold limit. The latter is defined as a urinary cadmium level greater than 3.1 μg/g creatinine.

Although urinary cadmium has been employed most frequently as a measure of exposure, both blood cadmium and plasma cadmium-metallothionein, determined by HPLC separation of plasma and reference to cadmium-metallothionein standards, have been used as well. Cadmium in whole blood has a biological half-life of 75-128 days for the fast component and 7.4 to 16.0 yr for the slow component. Thus blood cadmium can be used to monitor both recent and remote exposure. In current and previous occupationally- and environmentally-exposed individuals, a threshold blood cadmium level of 0.78 μg/l was determined. This stands in contrast to non-exposed individuals in whom the mean blood cadmium concentration was 0.36 μg/l.

Urinary excretion of N-acetyl-β-D-glucosaminidase (NAG), with a molecular weight of 120 kD, also has been reported to increase in humans exposed to cadmium. This is a lysosomal enzyme present in high concentrations in the proximal tubule. Because of its high molecular weight, its urinary origin is unlikely to occur via glomerular sieving but rather directly from damaged kidney tissue. Urinary NAG has been found to be elevated when urinary cadmium is below a threshold of 10 μg/g creatinine. Three thresholds have been noted: the first around 2 μg/g creatinine (associated with biochemical alterations), the second around 4 μg/g creatinine for high molecular weight proteins and some tubular antigens, and the third at 10 μg/g creatinine for low molecular weight proteins. Other indices of cadmium-induced renal dysfunction include increased excretion of tubular brush border antigens (BBA), thromboxane B, intestinal alkaline phosphatase, prostanooids, and glycosaminoglycans. Also found in the urine of both humans and animals intoxicated by cadmium is glutathione S-transferase (GST), a marker of tubular injury. The proteinuria induced by cadmium has been found to be irreversible, in general, even several years after cadmium exposure. However, microproteinuria may be reversible in some workers whose urinary cadmium exceeds a threshold of 10 μg/g creatinine but whose initial microproteinuria is mild (between 300 and 1,500 μg/g creatinine). The findings of generalized proximal tubular dysfunction are limited to workers with either clinically overt or subclinical tubular proteinuria.

Liver and kidney cadmium have been measured both at autopsy and in vivo, using neutron activation analysis. Roels et al examined workers in Belgian cadmium-producing plants and found a good correlation between liver cadmium and renal cortical cadmium, and between the log of urinary cadmium and renal cortical cadmium. The threshold level of renal cortical cadmium was between 200 and 250 ppm, in agreement with earlier estimates. In those workers with renal dysfunction there was a significantly higher liver
cadmium than in those with normal renal function, whose kidney cadmium concentrations were unchanged. This finding led the authors to speculate that renal cortical cadmium decreases progressively after the onset of kidney damage. The assumption was confirmed in another study when it was found in industrially-exposed workers that liver and kidney cadmium levels showed an increase until a concentration of 40 ppm was reached in the liver; thereafter, kidney levels decreased while liver levels increased.

Clinical manifestations

Glomerular filtration rate (GFR) impairment was not reported in early studies of cadmium workers. However, studies from Belgium found that workers with prolonged exposure (mean 25 yr) show a significant increase in serum creatinine and $\beta_2$-microglobulin with time, even when the effect of ageing is factored in. The authors suggest that prolonged cadmium exposure exacerbates the age-related decline in GFR. When these investigators assessed the filtration reserve capacity of the kidney (defined as the difference between baseline creatinine clearance and creatinine clearance after an acute load of protein), they found that cadmium workers with low molecular weight proteinuria had both diminished GFR and diminished filtration reserve capacity, whereas workers below age 50 without low molecular weight proteinuria showed neither abnormality. Although experimental evidence in rats has linked cadmium to hypertension, there is no conclusive evidence of such an association in humans. Mortality studies from cadmium-polluted areas in Europe and Japan have demonstrated that mortality rates for nephritis and nephrosis are higher than in control areas, although no increase in the incidence of cardiovascular deaths has been reported.

Itai-itai disease

The best characterized example of intoxication of a large population by environmental exposure to cadmium is itai-itai-byo or “ouch-ouch” disease, so named because of the crippling and painful osteomalacic component. The disease was endemic to the Jinzu River basin of the Toyama Prefecture in Japan, with peak incidence shortly after World War II, and was attributed to cadmium overload from a zinc mine upriver. The soil and the rice fields contained large amounts of cadmium brought with the irrigation water from the polluted river. Autopsies of five patients with itai-itai-byo revealed that the cadmium content of liver was 5 to 10 times higher than in age-matched controls, but the levels in kidney were lower than in the controls because kidney damage was present. A decrease in kidney cortex cadmium levels occurs with advancing age and with kidney disease of varying causes; this is consistent with decreased metallothionein content of the kidney cortex under these conditions.

The typical patient with itai-itai disease was reported to be a middle aged postmenopausal multiparous woman who had lumbar pains, leg myalgia, and ducklike gait. Pressure on bones, especially the femurs, spine, and ribs, aggravated the pain. Pseudofractures were seen on radiographic examination, and typical findings of osteomalacia were found on bone examination. Serum 1, 25-dihydroxy vitamin D (1$\alpha$, 25 (OH)\textsubscript{2}D) levels were decreased and were closely related to serum concentrations of parathyroid hormone (PTH), $\beta_2$-microglobulin, and renal tubular phosphate reabsorption. Quantitative histomorphometric measurements have been consistent with the clinical impression of osteomalacia. An interesting finding was the presence of iron at mineralization fronts, detected by radiographic microanalysis, suggesting a possible synergistic adverse effect between iron and cadmium on bone mineralization. Renal findings in these patients were typical of the Fanconi syndrome and included low molecular weight proteinuria, glucosuria, aminoaciduria, decreased tubular phosphorus reabsorption, low phenolsulphothalein excretion, and diminished concentrating ability; renal failure occurred in a few. In advanced cases, the kidneys were found to be contracted. Histologically, the kidney showed tubular atrophy and dilatation, eosinophilic casts, and interstitial fibrosis; glomeruli were normal.

Although the numbers of reported patients with full-blown itai-itai disease have been small and limited primarily to women, an epidemiological survey conducted in Japan revealed that about 45 per cent of women and 40 per cent of men older than 70 in the endemic area had concomitant proteinuria and glucosuria. Because the incidence of such abnormalities in younger subjects was not different from that seen in people living in a non endemic control area, it appears that tubular dysfunctions resulted either from the protracted exposure to cadmium from an early age or from some feature of ageing.

Other environmental exposure

Environmental exposure to lower limits of cadmium also can expose a health risk. An epidemiological survey of 2,327 subjects in two urban and two rural areas in
Belgium with different environmental pollution by cadmium revealed a probability of tubular dysfunction of 10 per cent when urinary cadmium levels reached 2 µg/day. There was a statistically significant dose-response relationship between the urinary cadmium excretion and five affected parameters: urinary excretion of retinol-binding protein, β2-microglobulin, NAG, amino acids, and calcium. No statistical association was found between environmental exposure to cadmium and blood pressure elevation.

Animal experiments

Experimental work in rabbits, rats, and mice indicates that renal functional or morphological changes appear only after the cadmium concentration in renal cortex reaches saturation levels of about 150 to 200 ppm wet weight (approximately 100 times normal). Repeated intraperitoneal cadmium administration in small dosages to rats produces renal tubular atrophy and interstitial fibrosis, and the first pathological alterations in cadmium-treated rabbits were seen in the proximal tubular epithelial cells. Cadmium is deposited chiefly in the renal cortex and is localized mainly in the proximal segment of the tubules. The metal deposits and renal tubular changes persist for several weeks after cessation of cadmium injections. Of particular interest is the observation that there is a long delay between initiation of cadmium injections in experimental animals and the first appearance of tubular dysfunction and increased urinary cadmium excretion. Anaemia in rats appears to be related to messenger ribonucleic acid (mRNA) hypoinduction for erythropoietin in the kidneys.

Role of metallothionein

The role of metallothionein, the low molecular weight cadmium-binding protein, is central to an understanding of cadmium nephrotoxicity. The purified protein was named metallothionein because of its high content of sulphur and metals, chiefly cadmium and zinc. Metallothionein synthesis probably occurs through formation of a specific mRNA because both cycloheximide and actinomycin D, administered concomitantly with cadmium, block the incorporation of [35S] cystine into cadmium-binding protein. Metallothionein can act as a transport protein, as well as a storage protein, for cadmium. When cadmium is injected together with metallothionein, there is a selective deposition of cadmium in the kidney, and the renal tubular damage produced is greater than that seen when cadmium is given alone. The increased renal damage can be attributed to the much more profound uptake of cadmium by the kidney when cadmium is given as metallothionein rather than free cadmium. The critical concentration of cadmium-metallothionein in kidney is 10 µg/g, in contrast to that of inorganic cadmium (130-200 µg/g). These observations are in accord with suggestions that cadmium bound to metallothionein is preferentially filtered through the glomeruli and subsequently reabsorbed and stored in tubules or enters the renal tubules directly from the peritubular capillaries. Zinc-metallothionein, in contrast to cadmium-metallothionein, is nontoxic when given in comparable dosage and appears to insert a protective effect against cadmium-metallothionein-induced nephrotoxicity.

Sato and Kondoh have published a review on the role of metallothionein in cadmium toxicity. Metallothionein synthesis is induced by various stimuli such as cadmium, mercury, zinc, oxidative stress, glucocorticoid, and anticancer agents. In recent studies using metallothionein-null mice, the ability of metallothionein to protect against cadmium-induced renal, liver and bone injuries has been confirmed. Metallothionein is also capable of scavenging oxygen free radicals. Unexpectedly, metallothionein-null mice were apparently in good health, raising a question as to the critical biological role of metallothionein. Experiments were performed in control and metallothionein-null mice, injected subcutaneously with a wide range of cadmium chloride doses, six times per week for up to 10 wk. In control mice, renal cadmium burden increased in a dose and time-dependent manner, reaching as high as 140 µg of cadmium per gram kidney, along with 150-fold increases in renal metallothionein concentrations, reaching 800 µg metallothionein per gram kidney. In metallothionein-null mice, renal cadmium concentration was much lower and renal metallothionein was nonexistent. Metallothionein-null mice were more susceptible than controls to cadmium-induced renal injury, as evidenced by increased urinary excretion of protein, glucose, gamma-glutamyltransferase, and NAG as well as by increased blood urea nitrogen levels. Kidneys of cadmium treated mice were enlarged and histopathology showed various types of lesions including proximal tubular degeneration, apoptosis, atrophy, interstitial inflammation, and glomerular swelling. These lesions were more severe in metallothionein-null than in control mice. Thus cadmium-induced renal injury was not necessarily mediated through the cadmium-
metallothionein complex, although metallothionein appears to be an important intracellular protein in protecting against chronic cadmium nephrotoxicity.

**Pathogenesis**

In our laboratory, an experimental model of cadmium-induced Fanconi syndrome was achieved by repetitive intraperitoneal administration of cadmium chloride in adult rats. Glucosuria and aminoaciduria were noted to appear abruptly after three weeks of injections, and the complete expression of the Fanconi syndrome (i.e., diuresis, glucosuria, and aminoaciduria, proteinuria, and increased sodium, potassium, calcium, magnesium, phosphate and urate clearance) was then elicited. At the peak of Fanconi syndrome, renal cortical adenosine 5’-triphosphate (ATP) levels were decreased by 38 per cent and cortex homogenate sodium-potassium-adenosine triphosphatase (Na-K-ATPase) activity was decreased by 60 per cent. Ultrastructural changes at this stage paralleled the biochemical findings in that the mitochondria were dramatically altered in size and shape and there was extensive loss of the basal plasma membrane infoldings together with complex cisternal proliferation in proximal tubular cells. The structural and biochemical findings were reversed within one week after cessation of cadmium injections despite persistence of total renal cortical cadmium concentration at levels close to 200 ppm wet weight. Analysis of subcellular fractions of the renal cortex revealed cadmium migration from the soluble cytoplasmic fraction (where metallothionein is located) to the mitochondrial fraction (where ATP is maximal) and to the microsomal fraction (where Na-K-ATPase activity is maximal) at the time of appearance of Fanconi syndrome, with reversal after cadmium injection discontinuation. Microsomal Na-K-ATPase activity decreased in tandem with the increase in microsomal cadmium concentration, and in vivo microsomal Na-K-ATPase inhibition was found to be precisely that predicted by in vitro titration of renal microsomal Na-K-ATPase with cadmium. When experimental animals were injected with [109Cd] 24 h before death and kidney cortex soluble cytoplasm was fractionated on Sephadex G-75, the radiolabeled cadmium was found to be associated principally with high molecular weight proteins in control animals but with the metallothionein fraction after cadmium injections were given. At the time of Fanconi syndrome development, the radiolabeled cadmium was again found mainly in association with high molecular weight proteins. These findings suggested that injected cadmium initially is segregated in a nontoxic complexed form as metallothionein in the cytoplasm of the proximal tubule, but when the organism’s capacity to synthesize new metallothionein is exhausted, the cadmium, now less firmly bound to high molecular weight proteins, moves to other cell organelles, where the ATP-Na-K-ATPase transport system is affected.

To further explore this possibility, animals were studied after 3, 6, 9, 14 (pre-Fanconi), and 15 (Fanconi) injections of cadmium. In addition, a post-Fanconi group was sacrificed 7 days after resolution of tubular dysfunction. Metallothionein was measured in liver and kidney cortex homogenates in each group of animals by tracing metallothionein with radioactive mercury as described by Zelazowski et al. Metallothionein concentration increased gradually from control values of 0.02 mg/g wet weight to a maximum of 2.90 mg/g wet weight in Fanconi animals, with relatively little further change in post-Fanconi animals. The binding capacity for [109Cd] of purified metallothionein from kidney cortex in each group of animals was assessed by Scatchard plot analysis. The number of binding sites were determined for both metallothionein and metal free thionein. These measurements were essentially unchanged in animals sacrificed at 3, 6, 9, and 14 days or in Fanconi animals. The number of binding sites in thionein was approximately 7 g atoms/mol and in metallothionein was about 0.6 g atoms/mol. The dissociation constant for thionein was 9 x 10⁻⁴ mol/liter and for metallothionein was 4 x 10⁻⁴ mol/liter. The cadmium and zinc content and the cadmium/zinc ratio were also determined in purified metallothionein from liver and kidney homogenates of pre-Fanconi and Fanconi animals. The most striking finding was a marked increase in the cadmium/zinc ratio in kidney metallothionein from Fanconi animals (50 in Fanconi animals versus 3 in pre-Fanconi)⁴⁴. Thus, the previously observed saturation level of cadmium in kidney cortex and the long delay after exposure before development of Fanconi syndrome may be ascribed both to a limit in the kidney’s capability of synthesizing metallothionein and the displacement by cadmium of zinc from common binding sites on the protein. The excess cadmium presented to the kidney spills over from the saturated protein-binding sites in the soluble cytoplasm to microsomal and mitochondrial fractions, where cadmium-sensitive enzyme systems are located. Thus, there is strong evidence that the ATP-Na-K-ATPase transport system is profoundly affected and appears to be the metabolic basis for altered transport in the proximal tubule.
An alternative, or perhaps complementary, explanation has been provided by Thevenod. Recent research has indicated that cadmium, as well as other heavy metals, induce oxidative damage in cells. Thevenod and Friedmann showed that cadmium chloride increased the production of reactive oxygen species in a rat proximal tubule cell line, as determined by oxidation of dihydrorhodamine 123 to fluorescent rhodamine 123. This was prevented by co-incubation with the thiol antioxidant N-acetylcysteine (NAC). Cadmium also increased apoptosis but not necrosis. Exposure of proximal tubular cells to cadmium decreased protein levels of the catalytic subunit of Na-K-ATPase, as determined by immunoblotting, by approximately 50 per cent, and NAC largely prevented this effect. In addition, the effect of quercetin, a potent reactive oxygen species scavenger, on metallothionein, nitric oxide synthases (NOS), and cyclooxygenase-2 (COX-2) renal expression following chronic cadmium administration to rats was studied by Morales et al. These studies indicated that renal eNOS expression was significantly higher in rats receiving cadmium and quercetin than in animals receiving cadmium alone or in control rats. In the rats that received cadmium, COX-2 and iNOS expression was markedly higher than in control rats. In the group given cadmium plus quercetin, no changes in COX-2 and iNOS expression were observed compared with the control group. In addition, animals receiving both cadmium and quercetin showed better renal function than those receiving cadmium alone. Total plasma antioxidants and renal SOD and glutathione-reductase activities were higher in the group that received cadmium and quercetin than in rats that received cadmium alone. Thus, in addition to direct inhibition of the ATP-Na-K-ATPase transport mechanism by cadmium, a second mechanism, namely stimulation of reactive oxygen species, may be operative in decreasing renal Na-K-ATPase activity, thereby reducing proximal tubular transport.

Immunologic renal disease

Because of the demonstrated ability of certain heavy metals, such as gold and mercury, to produce an immune complex nephritis, and because there is some evidence that cadmium-exposed workers developed glomerular lesions, studies have been conducted in experimental animals to determine whether cadmium can also induce immune complex nephritis. In one study, oral exposure of rats to either 100 or 200 ppm cadmium chloride in drinking water for 30 wk resulted in a diffuse membranous glomerulopathy with electron-dense deposits within mesangial cells on electron microscopy and granular dense deposits of immunoglobulin G (IgG) in most glomeruli by immunofluorescence. In another study of rats given 100 ppm cadmium chloride for up to 13 months, about 25 per cent of the rats had IgG glomerular deposits in the mesangial area. At 8 months, the rats had demonstrable circulating anti-laminin antibodies, suggesting that cadmium can induce autoantibody formation.

Treatment

Treatment for chronic or acute cadmium nephrotoxicity should be preventive. Once there is demonstrable renal disease, the person should be removed from all further exposure to cadmium. British anti-lewisite (BAL) should not be administered because there is evidence that the cadmium-BAL complex is more toxic to the kidney than cadmium alone. Of the chelating agents tested in experimental animals after acute oral cadmium intoxication, the most effective in both enhancing survival and leaving minimal residual levels of cadmium in liver and kidney was meso-2, 3-dimercaptosuccinic acid (DMSA, Succimer) and its esters. Carbodithioates have also proved to be effective in removing cadmium from rats. At present there is limited experience with the use of chelating agents, such as calcium disodium ethylenediaminetetraacetic acid (calcium EDTA), in treating acute or chronic cadmium poisoning in humans.

LEAD

Lead exposure sources

Lead is found in several industrial sources (Table I). Chief among these are the accumulator battery industry, lead smelters, lead or silver ore mining and lead refining. Non-industrial sources are air-borne lead from leaded gasoline fumes and lead-based paints. In the US lead was gradually removed from gasoline beginning in 1973, and was eliminated from gasoline in 1996. Lead in lead-based house paint was likewise eliminated in 1978. Because of these measures there was a gradual fall in mean blood lead values in the US population aged 1-74 from 12.8 µg/dl in the National Health and Nutrition Examination Survey (NHANES) II (conducted from 1976-1980) to 2.8 µg/dl in Phase 1 of NHANES III (conducted from 1988-1991). A further drop in mean blood lead occurred in the third phase of NHANES III (conducted from 1999-2002) to 1.64 µg/dl. Other countries, where lead controls on gasoline and paint have been less stringent, continue to
have evidence of low level lead intoxication. In Dhaka, Bangladesh, where one of the highest air lead levels in the world are found, the mean blood lead level in 779 school children studied in 2000 was 15.0 µg/dl. Even in Bombay (now Mumbai), India, where lead was removed from gasoline in 1997, blood lead levels in 754 children studied in 2002 yielded a mean values of 9.1 µg/dl. The lead content of paint samples from China, India, and Malaysia were found to contain 5000 ppm or more lead. This may account for the current scare in the US of painted toys imported from China. Other sources of lead include some Chinese and Indian herbal remedies and even candy. Ingestion of moonshine whiskey (distilled in car radiators) yields a distinctive form of lead nephropathy, described mainly from the southern US. Returning servicemen with retained bullets or shrapnel and employees or participants in indoor firing ranges are also exposed to lead. Finally users of skin products containing lead, such as litharge deodorant or kohl eye shadow, may absorb sufficient lead through the skin to become lead intoxicated.

### Lead dose measures

Lead in whole blood has a short half-life (35 days). Thus the use of blood lead measurements are restricted to monitoring concurrent lead exposure. For assessment of more remote lead exposure, other methods must be employed. Lead eventually settles in bone, where it comprises over 90 per cent of the total body lead burden in adults. Lead has a half-life in cancellous bone of 16 yr and in cortical bone of 27 yr. For this reason, measurements of the body burden of lead have been made by use of bone biopsy, non-invasive X-ray fluorescence of bone lead, or the chelation of lead by means of either calcium EDTA infusions or oral DMSA. The original studies of the calcium EDTA test in adults with childhood lead poisoning in Queensland, Australia, defined an abnormal test as >600 µg lead excreted in the urine 24 h after infusion of 1.0 g of calcium EDTA. Subsequently, the test collection was expanded to 72 h in patients with renal impairment. Batuman et al utilized this test to demonstrate that hypertensive patients with modest azotemia (serum creatinine > 1.5 mg/dl) had abnormal tests in contrast to hypertensive patients with normal renal function. The implication was that such patients had been intoxicated by lead at a remote time, with or without their knowledge. The oral DMSA chelation test has been used successfully in Korean lead workers to define the chelatable portion of the lead body burden. This test consists of administering DMSA in a dose of 10 mg/kg body weight and collecting the next 4 h urine.

The definition of an abnormal calcium EDTA test has gradually shifted. Lin et al from Taiwan have utilized repetitive calcium EDTA infusions in patients with predialysis chronic renal disease due both to diabetes and non-diabetes and have concluded that lead contributes to the progression of kidney diseases, and that chelation with calcium EDTA may slow or improve the deterioration of GFR. These authors initially defined an abnormal body lead burden as consisting of 80 to 600 µg lead following calcium EDTA; later, the definition was reduced to greater than 20 µg/72 h.

(Blood lead levels in Taiwan have been similar to those in the US, allowing for extrapolation of these conclusions). In a well-publicized study, Lin et al presented the results on 202 patients with chronic renal insufficiency (serum creatinine levels between 1.5 and 3.9 mg/dl). These patients were observed for 24 months and then they were randomly assigned to a control group and a group which received lead chelation therapy with calcium EDTA. For a three-month period the 32 patients in the chelation group received intensive lead chelation therapy. The mean change in GFR in this group was 2.1 ml/min per 1.73 m² body surface area as compared with -6 ml/min per 1.73 m² in the 32 controls.

Radiographic fluorescence techniques provide a noninvasive method of measuring lead at various bone sites. Both L-line radiographic fluorescence (L-XRF) and K-line radiographic fluorescence (K-XRF) have been used to measure lead at tibial and forefinger sites, representative of cortical bone, and patellar and calcaneus sites, representative of trabecular bone; most experience to date is with measurements at the midshaft of the tibia. The L-XRF technique uses energy of low
penetrating power and measures lead primarily in the outer 0.5 mm of bone. This measurement correlates well with the results of the calcium EDTA mobilization test. On the other hand, K-XRF, which uses higher intensity energy (derived from radioisotopes such as \(^{57}\)cobalt or \(^{109}\)cadmium), measures the concentration of lead throughout the bone thickness. The radiographic fluorescence techniques have been used successfully for epidemiological studies of lead exposure, as an index of cumulative exposure in occupationally exposed patients, as a measure of success of lead removal by chelation in lead-intoxicated children, and as a way to verify remote lead exposure in patients with suspected chronic lead nephropathy. By means of the K-XRF test, it was established that the mean tibia lead was 20.8 \(\mu g/g\) bone mineral in 719 participants in the Boston, MA Normative Aging Study. These men had environmental but not occupational exposure to lead. Mean tibia bone lead in Korean lead workers was 37.2 \(\mu g/g\).

Measurement of enzymes in the blood that are sensitive to lead, have also been used to follow both acute and remote lead intoxication. Fontanellas et al from Spain explored the use of the ratio of delta-aminolevulinic acid dehydratase (ALAD) to restored ALAD (restored by adding zinc and dithiothreitol to blood) in a number of patients with chronic renal failure at a pre-dialysis stage, dividing them into two groups after the EDTA mobilization test had determined whether lead pools were expanded. The study included 24 healthy controls, 12 patients with clinical plumbism and biochemical demonstration of lead poisoning, 18 patients with chronic renal failure with no evidence of high lead storage, and 8 patients with chronic renal failure with high urinary excretion of lead (greater than 600 \(\mu g/72\) h after calcium EDTA). The ALAD/restored ALAD ratio correlated closely with urinary lead excretion. It was normal in healthy controls, and in the chronic renal failure patients without excessive urinary lead but was significantly reduced in the patients with clinical plumbism and in the chronic renal failure patients with increased excretion of lead in their urine. It should be noted, however, that this study was carried out in Spain at a time when the mean blood lead value was 18 \(\mu g/dl\).

**Susceptibility to lead intoxication**

There has been considerable interest in determining the factors that dictate individual susceptibility to lead. Lead in whole blood resides primarily in the erythrocyte (99%), wherein it is bound primarily to a 240 kD protein, identified as the enzyme, ALAD. An additional factor is the inducible 10 kD erythrocyte lead-binding protein found in lead workers, which appears once blood lead exceeds 39 \(\mu g/dl\) and seems to bind lead in a non toxic form. A subset of workers who are incapable of responding to lead exposure by mounting a response in the 10 kD lead-binding protein demonstrated evidence of toxicity at low blood lead levels. A similar phenomenon has been described in the genotypic polymorphism of ALAD. The ALAD gene contains two codominant alleles: ALAD-1 and ALAD-2. ALAD-1 is the predominant allele. Workers who are heterozygous or homogyzous for ALAD-2 have blood lead values higher than those of similarly exposed workers who are homozygous for ALAD-1. The difference apparently results from the greater propensity for ALAD-2 to bind lead in a non toxic form. Less bone lead, urinary delta-aminolevulinic acid, zinc protoporphyrin, and DMSA-chelatable lead (all markers of lead accumulation or toxicity) are found in ALAD-2 patients.

**Clinical manifestations of lead intoxication**

In the early 20th century, there were several reports of industrially related progressive renal failure from lead poisoning. Initial reports were scanty, suggesting the possibility that improved working conditions and medical surveillance might have aborted the development of significant renal disease. However, a 1968 study of 102 industrially exposed workers in Romania demonstrated that 17 had evidence of renal impairment when tested by discrete renal function tests such as creatinine clearance or urea clearance. A subsequent epidemiological survey of American lead workers revealed that after adjustment for the effect of age, there was a direct correlation of both serum creatinine and blood urea nitrogen (BUN) with the length of lead exposure. Another survey of American lead workers in 1979 found that GFR, as measured by iothalamate-125 clearances, was reduced in 21 of 57 lead workers in whom excessive body lead burdens had been shown by the urinary excretion of more than 1,000 \(\mu g/dl\) per day during a calcium EDTA lead mobilization test. A Swedish study of five lead workers with different periods of lead exposure revealed that despite an abnormal GFR in only one of five, all had abnormal renal biopsies. Proximal tubule nuclear lead inclusion bodies were found in the biopsies of two workers who had less than one year’s exposure to lead, whereas the biopsies of the three subjects with exposures of four to more than 12 years did not contain these inclusions, but showed varying degrees of diffuse interstitial or peritubular fibrosis.
The presence of lead-induced renal disease may relate to the severity of the body burden of lead. A 1992 survey of 70 active and 30 retired Swedish lead smelter workers found no evidence of renal abnormalities, as measured by plasma creatinine, creatinine clearance, β₂-microglobulin clearance, urinary albumin, or urinary excretion of the enzyme NAG, compared with a control cohort. The mean blood lead values in these workers had gradually diminished from 63 to 34 µg/dl from 1950 to 1987 as controls over lead exposure improved. Although the relationship between body burden of lead and development of chronic lead nephropathy has not been defined strictly, a World Health Organization Task Group concluded that prolonged lead exposure with blood lead levels greater than 70 µg/dl can result in chronic irreversible nephropathy. Studies of low lead exposure in industry (blood lead consistently below 60µg/dl) have failed to show convincing evidence of nephropathy. However, an epidemiologic survey of a Belgian population with known environmental exposure to both lead and cadmium (blood lead range 1.7 to 72.5 µg/dl, geometric mean 11.4 µg/dl) revealed an inverse correlation between blood lead and creatinine clearance after adjustment for age and body mass index. A ten-fold increase in blood lead was associated with a creatinine clearance reduction of 10 to 13 ml/min. Similar findings were reported by Payton et al in the Normative Aging study of Boston veterans, where the mean blood lead level was 8.1 µg/dl. In children, the generally accepted blood lead value which signifies a potential hazard is 10 µg/dl. Values above this level are reportable. The lower limit of blood lead, below which no toxicity is observed, has not been established.

In addition to occupation-related disease, two unusual forms of lead nephropathy have been described in adults, one related to remote childhood lead exposure and the other to illicit moonshine whiskey ingestion. In both situations, there has been a high incidence of saturnine gout. Evidence indicates that a large proportion of the cases of chronic renal failure in adults in Queensland, Australia, was attributable to childhood lead poisoning. There was an unusually high incidence of acute lead poisoning in children around the beginning of the 20th century related to ingestion of large quantities of lead-based paint, deposited as flakes in the dirt surrounding the verandas of their homes. Twenty or more years later, many of these children developed chronic renal failure, with granular contracted kidneys. Lead was confirmed as the cause when the bone lead content of these patients with cryptogenic kidney disease was found to be significantly greater than in other patients with chronic nephropathy of recognized cause. Subsequent studies used calcium EDTA to mobilize lead from bone. Urinary lead excretion after 1 g calcium EDTA invariably exceeded 600 µg/day in patients with renal insufficiency attributable to childhood lead poisoning, whereas lead excretion was below this figure in normal controls and in the majority of patients with renal insufficiency resulting from causes other than lead.

Although the Queensland experience seems incontrovertible, attempts to confirm that childhood plumbism leads to adult renal insufficiency in other parts of the world have been unsuccessful; the difference may lie in the degree of childhood lead exposure or factors influencing the subsequent mobilization of lead from bone. Indeed, in a 50-year follow up of childhood plumbism in the United States, studies comparing 21 lead-exposed subjects with age-, sex-, race-, and neighbourhood-matched controls revealed supernormal creatinine clearance in the lead exposed subjects, who also had a higher risk of hypertension. On the other hand, a longitudinal study of low level lead exposure, determined by repetitive blood lead measurements in 459 men during the Normative Aging Study, found that blood lead concentration was significantly and positively associated with serum creatinine concentration; a ten-fold increase in blood lead predicted an increase of 0.08 mg/dl in serum creatinine. Furthermore, there was an acceleration of age-related changes in renal function in association with long-term low lead exposure.

A chronic lead nephropathy remarkably similar to the Queensland variety has been described in moonshine whiskey drinkers in the southern United States. The renal pathological findings were similar to those seen in the Queensland cases, and intranuclear inclusion bodies were found commonly. A diagnostic infusion of calcium EDTA provided confirmatory evidence of excessive lead storage in these subjects. In addition to the high incidence of hyperuricaemia and gout mentioned earlier, these patients also have unusually high rates of salt wasting, acidosis and hyperkalaemia. These findings may be attributable to hyporeninemic hypoaldosteronism, subsequent to lead intoxication, as well as to inhibition of distal tubular Na-K-ATPase.

The incidence of lead related end-stage renal failure in the general adult population is unknown, although attempts to estimate the incidence have been made.
through measurements of bone lead in patients on dialysis. In one study, lead and lead: calcium ratios were measured in trans- iliac bone biopsies obtained from 153 patients on dialysis from units in several European countries. Elevated bone lead was found in 5 per cent of the patients on haemodialysis, approximating levels found in active workers (20 µg/g in the patients on dialysis, 30 µg/g in Belgian lead workers, and 6 µg/g in normal subjects). Bone lead concentrations in patients with analgesic nephropathy receiving dialysis were comparable to control measurements in deceased subjects who had normal renal function, indicating that neither end-stage renal failure nor dialysis treatment contributes to the total body burden of lead. What remains unclear is whether the 5 per cent incidence of elevated bone lead in the patients on dialysis represents a 5 per cent incidence of lead-induced chronic renal failure or whether 5 per cent of patients on dialysis had increased exposure to and thus retention of lead, with the lead either playing no role or contributing to progressive renal failure initiated by other causes.

**Relationship to gout**

Covert lead poisoning may be responsible for sporadic cases of pre-end-stage renal failure gouty or hypertensive nephrosclerosis. In separate studies conducted in the United States, Germany and Australia, and Italy, higher urinary lead excretion after calcium EDTA mobilization was seen in patients with gout and moderate degrees of renal failure than in patients with no gout but comparable degrees of renal impairment. Elevated urinary lead was less common in patients who had gout before renal failure than in those who presented with renal failure before gout. Another study from Spain of 297 subjects (30 normal controls, 105 patients with essential hypertension, 132 patients with chronic renal failure and hypertension or gout, and 30 patients with chronic renal failure of known cause) revealed that groups II and III had abnormal calcium EDTA tests (15.4% in group II and 56.1% in group III).

From a functional standpoint, lead nephropathy often is accompanied by hyperuricaemia and reduced renal excretion of urate, sometimes in association with overt gouty arthritis. The pyrazinamide suppression test initially demonstrated that this constellation of findings is caused by an enhanced tubular reabsorption of urate rather than by reduced secretion. However, subsequent studies revealed that urate handling by the kidney was a four-component system, namely filtration, pre-secretory reabsorption, secretion, and post-secretory reabsorption. In clinical gout there is diminished secretion of uric acid, most likely this occurs in lead nephropathy accompanied by hyperuricaemia as well. Lin et al have demonstrated that gouty patients are more likely to have “high normal” lead excretion after calcium EDTA infusions compared to normals without gout and that such infusions in patients with gout increase urate excretion and lower serum uric acid, further confirming the relationship between gout and lead intoxication.

**Diagnosis of lead nephropathy**

The clinical characteristics of lead nephropathy are listed in Table II. Unlike in most other renal diseases, proteinuria is absent or minimal and the urinary sediment is benign. The presence of renal disease is recognized primarily by abnormalities in overall renal function (i.e., elevated BUN or serum creatinine concentration and decreased urea or creatinine clearances). Detection improves when discrete renal function tests are used, such as inulin (or iodothalamate-125 or chromium-51 EDTA) clearance measurements of GFR or p-aminohippurate (PAH) clearance measurements of renal blood flow. As indicated earlier, lead nephropathy resulting from ingestion of moonshine whiskey also can show a greater propensity toward salt wasting, acidosis, and hyperkalaemia than other renal diseases with a comparable degree of renal failure. Expression of elements of proximal tubule dysfunction is seen primarily in children who have had acute high-level lead poisoning. Overt manifestations of proximal tubular dysfunction are rare in adults with lead nephropathy, although one study demonstrated a

<table>
<thead>
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<th>Table II. Clinical and pathologic abnormalities of lead nephropathy</th>
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<tr>
<td>Reduced glomerular filtration rate</td>
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<td>Reduced renal blood flow</td>
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<tr>
<td>Absent or minimal proteinuria</td>
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<td>Normal urine sediment</td>
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<td>Variable hypertension</td>
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<tr>
<td>Hyperuricaemia, low urate clearance, and gout common</td>
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<td>Acidosis and hyperkalaemia sometimes seen</td>
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<tr>
<td>Increased urinary excretion of the enzymes NAG, GST and α₁-microglobulin</td>
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<td>Proximal tubular dysfunction seen only in acutely intoxicated children</td>
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<td>Interstitial nephritis and proximal tubular nuclear inclusion bodies on renal biopsy</td>
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reduced glucose reabsorption capacity, but normal bicarbonate reabsorption capacity and normal PAH reabsorption capacity in four occupationally exposed workers with minimally reduced GFR. Attempts to develop markers of renal dysfunction in patients with early lead nephropathy (e.g., in the industrial setting) have met with variable success. Not only is albuminuria absent in early stages of renal disease, but $\beta_2$-microglobulinuria, which has proved to be a very useful marker of cadmium nephrotoxicity, also is absent in lead-induced renal disease. The urinary excretion of certain renal tubular lysosomal enzymes, such as NAG and lysozyme, has been found to be elevated in some lead workers. A collaborative European study examined more than 20 potential indicators of renal changes in 50 workers exposed to lead (with average blood lead 48 $\mu$g/dl) and found that the most outstanding effect was a lowered urinary excretion of the eicosanoid 6-keto-PGF$_{1\alpha}$, and enhanced excretion of thromboxane (TXB$_2$). In another study of 81 exposed workers with a mean blood lead of 42 $\mu$g/dl, enhanced 6-keto-PGF$_{1\alpha}$ and TXB$_2$ excretion was found, together with enhanced intestinal alkaline phosphatase, brush border antigen (BBA) and prostaglandin PGF$_{2\alpha}$ and PGE$_2$ excretion, together with decreased fibronectin excretion. The authors claim that glomerular injury is heralded by the alterations in 6-keto-PGF$_{1\alpha}$, TX B$_2$, and fibronectin, proximal tubular injury by intestinal alkaline phosphatase and BBA, and collecting tubule or medullary interstitial cell injury by PGF$_{2\alpha}$ and PGE$_2$.

In animal studies only glutathione-S-transferase (GST) and $\alpha_1$-microglobulin seemed to be appropriate urinary markers. NAG, which has been most extensively investigated in humans, appears to be upregulated early rather than a response to tubular injury, increasing in low lead treated animals despite an absence of pathological changes on ultrastructural study (vide infra). $\beta_2$-microglobulin, and possibly retinol binding protein, which are low molecular weight proteins reabsorbed by the proximal tubule, appeared to be elevated only with high levels of blood lead (> 80 $\mu$g/dl). None of these markers are specific for lead.

Pathology of lead nephropathy

The gross pathologic finding of chronic lead nephropathy is that of a granular contracted kidney. Tubular atrophy and dilatation with interstitial fibrosis, but minimal cellular infiltration, typically are seen. Glomeruli become sclerotic secondary to blood flow impairment, but there is no evidence of a primary glomerulonephritis of immune pathogenesis. Vascular lesions, with intimal proliferation and hyaline degeneration of the media, may be prominent. The eosinophilic proximal tubular nuclear inclusion body, once thought to be pathognomonic of lead nephropathy, is present only during the early years of lead exposure. Thus, the diagnosis of lead nephropathy usually is inferential, based primarily on a history of lead exposure, evidence of renal impairment by functional testing, and renal biopsy disclosure of a non immunologically mediated interstitial nephritis.

Sanchez-Fructuoso et al evaluated pathological changes as well as the response of ALAD activity before and after calcium EDTA treatment in rats fed 500 ppm lead acetate for 90 days. In the lead-treated animals the main findings were hypertrophy and vacuolization of medium and small arteries, mucoid oedema and muscular hypertrophy in arterioles, loss of cell brush borders, cell loss, and intranuclear inclusion bodies in the proximal tubule, and fibrosis and the presence of infiltrates in the interstitial component. Treatment with calcium EDTA slowed the progression of most alterations and resulted in a diminution in nuclear inclusion bodies. ALAD activity was reduced in the lead exposed rats but was restored in the rats treated with calcium EDTA.

Animal studies

An experimental model of lead nephropathy has helped to elucidate the effects of lead on the function and ultrastructure of renal proximal tubule lining cells. Moore and colleagues were principally responsible for defining the role of renal proximal tubular nuclear inclusion bodies (a lead-protein complex) in the response to lead intoxication. The main component of the protein in the inclusion body has an approximate molecular weight of 27 kD or 32 kD, that is rich in glutamate and aspartate and contained about 40 to 50 $\mu$g lead per milligram of protein. Isolated mitochondrial preparations from these animals have been demonstrated to have impaired oxidative and phosphorylative abilities compared with mitochondria from control rats. When lead-poisoned rats were treated with EDTA, the nuclear inclusion bodies were disrupted and removed from the nuclei concomitant with an increased urinary lead excretion. Kidney cytosol has been shown to contain 3 lead-binding protein fractions-11.5, 63, and >200 kD. Only the 11.5 kD fraction was capable of reversing lead-induced ALAD inhibition in liver homogenates.
Our laboratory has developed long-term models of both low and high lead nephropathy in male Sprague-Dawley rats fed a low calcium diet\textsuperscript{123–125}. Lead acetate was used in concentrations of 0.5 per cent (high dose) and 0.01 per cent (low dose) in drinking water for periods from 1 to 12 months and lead-exposed animals were compared to pair-fed control rats. In all studies GFR was measured as\textsuperscript{125}$^{125}$I-iothalamate clearance by a single injection technique. Urinary markers included NAG, GST, and brush border antigens (BB50, HF5, and CG9) and were expressed as units/g creatinine. Blood and urine lead were measured prior to sacrifice in each group of animals. Kidneys were processed for light, electron and immunofluorescent microscopy. Animals treated with continuous high dose lead for 12 months reached a maximum blood lead of 125.4 ± 10.1 µg/dl after 6 months, at which time the dose of lead was reduced from 0.5 to 0.1 per cent\textsuperscript{123}. Blood lead at the end of 12 months averaged 55 µg/dl. Urine lead remained above 100 µg/g creatinine at all times but was highest at 3 months, averaging 340 µg/g creatinine. In the lead-treated animals, GFR was increased above controls at 3 months (1.00 ± 0.14 vs 0.83 ± 0.26 ml/min/100 g body wt, \textit{P}=0.05), then declined after 6 months to 0.78 ±0.16 vs 0.96 ± 0.08 ml/min/100 g body wt in controls. With regard to urinary markers, both NAG and GST were elevated above control values after 3 months of lead exposure. No significant differences were observed in brush border antigens. Pathology examination revealed that proximal tubular nuclear inclusion bodies were present at all time periods in lead-treated animals. Enlargement of proximal tubular cells and nuclei were seen beginning at 3 months. At 6 months, focal tubular atrophy and interstitial fibrosis appeared, increasing in extent up to 12 months. Mitochondrial alterations, consisting of rounding and elongation, appeared by 1 month and were persistent. Glomeruli were normal through 9 months, but at 12 months showed focal and segmental sclerosis. There were no electron dense deposits and immunofluorescent studies were negative. Renal arteries and arterioles were normal at all times.

In the second study\textsuperscript{124} the course of events was examined over 12 months in continuous low level lead-exposed animals. Maximum blood lead levels in experimental animals were reached at three months, averaging 29.4 ± 4.1 µg/dl. GFR was found to be significantly increased above pair-fed controls at 1 and 3 months, but was normal at other time periods (1 month experimental, 1.18 ± 0.12 vs control, 0.76 ± 0.15 ml/min/100 g; \textit{P}<0.001; 3 month experimental, 1.12 ± 0.16 vs control, 0.86 ± 0.10 ml/min/100 g.; \textit{P}<0.001). Levels of urinary NAG exceeded levels in controls at essentially all time periods, whereas urinary GST, a more specific marker of metal-associated proximal tubular injury, was normal. Proximal tubular nuclear inclusion bodies were sparse and were observed only at 1 and 3 months. There were no other pathological alterations in the kidneys up to 12 months, when mild tubular atrophy and interstitial fibrosis were seen. The absence of changes in urinary GST accorded with the relative absence of morphological changes, whereas the observed increase in urinary NAG suggests that this enzyme may be an overly sensitive indicator of tubular injury, more probably reflecting upregulation of the enzyme in the absence of tubular injury. It should be noted that both low dose lead-treated and high dose lead-treated animals showed a “hyperfiltration” phenomenon during the first 3 months of lead exposure. Thus these studies join those in humans\textsuperscript{81,97,126} which indicate that lead nephropathy should be added to diabetic nephropathy as diseases which may lead to hyperfiltration early in the course.

A third study\textsuperscript{125} consisted of discontinuation of both high and low dose lead after 6 months, then treatment with 3 courses of DMSA or discontinuation of high dose lead alone after 1, 6 and 9 months of lead feeding in rats. Controls were pair-fed, exposed to lead for six months, then removed from exposure for six months without receiving the DMSA. Low dose lead-treated rats showed no significant pathological changes with or without DMSA treatment but exhibited a significant increase in GFR after DMSA (1.09 ± 0.19 vs 0.88 ± 0.22 ml/min/100 g body weight; \textit{P}<0.03). Urinary markers remained unchanged, and there were no structural alterations by light or electron microscopy. High dose lead-treated animals showed no functional or pathological changes when lead exposure was discontinued after one month. However, when duration of exposure was six or nine months, GFR was decreased and serum creatinine and urea nitrogen were increased as compared to controls. Tubulo-interstitial disease was severe. Administration of DMSA resulted in an improvement in GFR and a decrease in albuminuria, together with a reduction in size and number of nuclear inclusion bodies in proximal tubules. However, tubulo-interstitial scarring was only minimally reduced. Treatment with the chelator, DMSA, improved renal function, but had less effect on pathological alterations. As GFR improved after DMSA treatment in both low
dose and high dose lead-treated animals, irrespective of the degree of pathological alterations, it may be concluded that the DMSA effect is most likely mediated by haemodynamic changes.

**Pathogenesis**

As with other heavy metals, lead administration leads to accumulation of reactive oxygen species, and treatment with scavengers results in lowering of blood pressure and improvement in GFR. A sequence of reactive oxygen species can be demonstrated in lead-induced disease, i.e., an increase in superoxide radical, hydroxyl radical, hydrogen peroxide, and peroxynitrite, together with a diminution in GSH in liver, brain, and aorta. Nitric oxide is most commonly decreased (by reactive oxygen species) as is urinary cyclic GMP. Aortic guanylate cyclase is decreased. The enzyme responsible for an increase in the production of free radicals, NAD(P)H oxidase, is increased by lead, whereas eNOS and iNOS, the enzymes involved in production of nitric oxide, are also increased, attesting to the importance of free radical destruction of nitric oxide rather than decreased formation. Antioxidants reverse these changes and diminish blood pressure. Various antioxidants have been used in conjunction with chelators, to both remove lead from tissue and to diminish free radicals. N-acetyl cysteine, taurine, lipoic acid, arginine, vitamin C, vitamin E, thiamine, tempol, and lazaroids all improve free radical diminution.

Captopril, an angiotensin-converting enzyme inhibitor, acts as an antioxidant in lead-exposed rats. In one study lead acetate was given in water for six weeks; captopril-administered animals received captopril (10 mg/day) during the sixth week. Blood lead in the control group measured 0.8 µg/dl vs. 23.8 ±1.6 µg/dl, in the lead plus captopril group 24.6 ± 20 µg/dl. MDA concentrations (a measure of reactive oxygen species) in liver, brain and kidney were increased by lead administration and reduced to or towards normal by lead plus captopril treatment.

Fox et al explored the effect of lead exposure in vivo on adult rat retinal and kidney Na-K-ATPase. Pups, exposed to lead through the milk of dams consuming zero, 0.02 or 0.2 per cent lead solutions, had mean blood lead concentrations of 1.2, 18.8, and 59.4 µg/dl at weaning, respectively, and 5-7 µg/dl as 90-100 day old adults. Prior lead exposure produced significant dose dependent decreases in isolated retinal Na-K-ATPase activity whereas activity in the kidney was unchanged (? effect of lead-binding proteins). In contrast, Na-K-ATPase from both isolated control tissues was inhibited by lead in vitro. The half-maximal inhibitory dose of lead for retinal and renal Na-K-ATPase was 5.21 x 10⁻⁷ and 1.25 x 10⁻⁵ M, respectively. Kramer et al had also explored the half-maximal inhibitory dose for lead chloride on renal cortical homogenate Na-K-ATPase. This was found to be 7 x 10⁻⁴ M. There was a competitive inhibition by lead with regard to the substrate, ATP.

**Treatment**

Acute lead intoxication without renal involvement or lead nephropathy ordinarily is treated with EDTA chelation. Although sodium EDTA has been shown to have toxic propensities because of its calcium chelation properties, calcium EDTA in appropriate dosages is useful and relatively harmless. The usual recommendation is for a course of 5 to 7 consecutive days in a dosage of 50 mg/kg/day or less, and an administration rate of 20 mg/min or less. A common practice is to use 1.0 g calcium EDTA in 250 ml normal saline, delivered intravenously over 2 h. No adverse side effects have been reported with this technique. A small number of cases of acute tubular necrosis have been described with sodium or calcium EDTA therapy, related mainly to very large dosages, rapid administration, or severe pre-existing renal or metabolic disease. Trace metal depletion, although of theoretical concern, has not yet been shown to be clinically important, although zinc excretion is known to be markedly elevated by calcium EDTA. Advanced renal disease related to lead intoxication (GFR less than 50% of normal) must be treated cautiously, because EDTA is filtered by the glomerulus, much as inulin is. In such instances, the dosage and infusion rate of EDTA should be reduced in proportion to the serum creatinine elevation. Lead nephropathy should be treated energetically, however, because treatment may stabilize or improve renal function.

Penicillamine can be used as an oral chelating agent, but only when the worker has been totally removed from a lead environment, because this agent can enhance gastrointestinal absorption of ingested lead. It is less effective as a chelator than calcium EDTA, and, with prolonged administration, can produce renal toxicity (nephrotic syndrome), leukoopenia, and anaemia. Two new oral chelating agents, dimercaptopropane sulphonate (DMPS) and DMSA, water-soluble derivatives of BAL, have been shown to be effective.
lead chelators in experimental animals. DMSA has been approved for treating children with lead intoxication and is effective in lead workers.

Conclusions

Both cadmium and lead are nephrotoxic and can lead to progressive renal failure. While cadmium toxicity presents as a generalized disorder of proximal tubular function, including increased uric acid clearance and hypouricaemia, lead toxicity (except in children) is commonly accompanied by hyperuricaemia and/or gout. Markers for early renal disease reflect these differences. Cadmium nephrotoxicity is heralded by increased excretion of proteins and enzymes in workers exposed to cadmium. Experimental confirmation in rats of the mixed type proteinuria observed in workers exposed to cadmium. Toxicol Lett 1985; 24:195-201.

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