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Alterations in rabbit kidney protein expression following lead exposure as analyzed by two-dimensional gel electrophoresis

It was recently reported that low blood lead levels impaired kidney function in men. To develop a set of molecular markers of renal lead exposure and effect, we investigated changes in renal protein expression while approximating occupational lead exposure at subchronic, low blood levels. Lead was administered to male Dutch Belted rabbits as a lead acetate solution adjusted weekly to achieve and maintain the target blood lead levels of 0, 20, 40, and 80 µg/dL for 15 weeks. Lead exposure did not affect kidney or body weights. The effect of increasing blood lead on protein expression was evaluated in rabbit kidney by large-scale two-dimensional electrophoresis (2-DE). Significant quantitative changes ($p < 0.05$) occurred in a dose-related manner in 12 proteins at 20 µg/dL exposure, 25 at 40 µg/dL, and 102 at 80 µg/dL. At a higher level of significance ($p < 0.001$), 40 µg/dL blood lead resulted in one protein alteration and 80 µg/dL affected 14 proteins. A set of quantitatively altered charge variants was tentatively identified as glutathione-*S*-transferase (GST), based on similar observations in rodents subjected to short-term, very high lead exposure. The significance of the protein alterations observed as markers of toxicity awaits their conclusive identification. Investigation of the kidney 2-DE profile in lead-exposed rabbit may be useful in understanding the mechanism of lead nephrotoxicity in humans.

Keywords: Rabbit kidney / Lead toxicity / Two-dimensional polyacrylamide gel electrophoresis
EL 3625

1 Introduction

According to the National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health (NIOSH), 827 000 American workers are potentially exposed to lead [1]. Lead is a common occupational and environmental hazard because of its broad industrial use in the manufacturing of batteries, fuel additives, pipes, pigments, solders, and shielding. Excessive exposures occur most frequently in nonferrous foundries, radiator repair shops, firing ranges, lead smelters, construction sites, and demolition sites. Uptake of lead occurs mostly by inhalation, although ingestion may contribute to exposure. The kidney, a primary target organ in the initial accumulation of absorbed lead [2], takes it up through glomerular filtration, tubular reabsorption, and a small fraction through direct absorption [3–6].

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Abbreviations: GST, glutathione-*S*-transferase; **MSN**, master spot number

Lead-induced nephrotoxicity in workers and experimental animals is well-documented and characterized as decreased glomerular filtration rate and nephropathy of proximal tubules [7, 8]. Kim *et al.* [9] reported increasing blood lead levels even within a low-range impaired kidney function in adult men. In carcinogenesis studies, 45% of male Sprague-Dawley rats developed kidney tumors after 58 weeks of a 1% lead acetate diet [10]. Body weight decreased to 80% of the control and the organ/body weight ratio of kidney and liver increased to 200% and 114% of the control levels, respectively. Choie and Richter [11] reported that a single dose of lead acetate (0.04 mg/g body weight) stimulated a remarkable replication of proximal tubular cells in rat kidney in two days. Rabbits and humans share many similarities in the luminal lead load of renal tubular cells and haemsynthesis pathway response to lead intoxication [12]. It has been suggested that the luminal lead load of renal tubular cells may be very similar in man and rabbit [13], because of the similarity in the single nephron glumerular filtration rate and the average length of the proximal tubules in humans and rabbits. We have developed an experimental rabbit model with sustained blood levels comparable to those found in occupational settings (20–80 µg/dL). As part of a multidisciplinary research project to evaluate the rabbit as a toxicological model for lead exposure, we investigated the effect of elevated blood lead on rabbit renal two-

Proteomics and 2-DE

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dimensional gel (2-DE) protein profiles. Using 2-DE and computerized image analysis, we resolved the complex mixture of hundreds of cellular proteins and mapped phenotypic alterations in lead-treated rabbit kidney cells. These studies provide a preliminary mechanistic understanding at the biochemical level of the lead-induced toxic effect on the kidney glomerulus and proximal tubules and offer insight into the putative basis for lead nephrotoxicity in the exposed worker.

2 Materials and methods

2.1 Animal care

Sexually mature male Dutch Belted rabbits (approximately 6–7 months of age, 2 kg) were obtained from Hazelton Research Products (Aberdeen, MD). They were individually housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved animal facility (19–21°C) in stainless steel (25″ × 20″ × 16″) cages as required by the current United States Department of Agriculture (USDA) guidelines. All rabbits were individually marked/identified and were observed daily. A 12 h light/dark cycle was maintained. Each rabbit was fed certified High Fiber Purina (Nr. 5325) rabbit chow (limited to 125 grams/day) and water *ad libitum*. Trace analyses including lead and pesticides were conducted on food and water.

2.2 Treatment and blood collection

The lead treatment protocol consisted of a 5-week predosing period and a 15-week dosing period [14]. Following the baseline period the rabbits were randomized into a control and three dose groups (n = 15/group). Lead was administered as a lead acetate solution in sterile 5% dextrose *via* subcutaneous injection to circumvent dietary effects on gastrointestinal absorption and allow enhanced control of blood lead concentrations. The controls were given a subcutaneous injection of 5% dextrose. Each rabbit was given 3 mL of the loading dose solution three times per week during study weeks 6–10. Animals were dosed on a mg/kg basis as follows: 260, 360, or 1300 μg/kg lead to achieve targeted blood levels of 20, 40 or 80 μg/dL, comparable to those lead levels achieved in the exposed worker. Following the loading dose period, rabbits were then placed on maintenance dose solutions during study weeks 11–20. The concentration of the maintenance dose solution administered was calculated using the previous week's blood lead value as a percentage of the expected dose. Blood lead levels were determined weekly throughout the entire 20-week period. Blood was collected by jugular phlebotomy, added to 1 mL EDTA (ethylenediaminetetraacetic acid) solution, and analyzed for lead by GFAAS-Z (graphite furnace atomic absorption spectroscopy with Zeeman background correction) [15]. As quality control checks, reference blood pools for instrumental calibration along with centers for Disease Control and Prevention (CDC) certified blood pools were included. The limit of detection was determined to be 0.1 μg/g.

2.3 Collection of rabbit kidney

Animals were injected intravenously with 1250 IU heparin sodium *via* the marginal ear vein at least 20 min prior to sacrifice. Animals were weighed, then sacrificed by overdose with a concentrated solution of sodium pentobarbital (approximately 75 mg/kg) *via* the marginal ear vein. Major organs, *i.e.*, the brain, liver, gall bladder, accessory sex organs as well as kidney, were evaluated for pathological changes. A 250 mg piece of kidney was sectioned (coronally) at or near the cortico-medullary boundary such that each sample was composed primarily of renal cortical cells. Kidney sections were removed and stored at –70°C.

2.4 Two-dimensional gel electrophoresis

Tissue samples were minced, ground-glass homogenized and solubilized in 2 mL of a lysis buffer (pH 9.5) containing 9 M urea (BDH Chemical, Poole, UK), 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; Calbiochem, La Jolla, CA), 1% dithiothreitol (DTT; Sigma, St. Louis, MO) and 2% carrier ampholytes, pH 8–10.5 (Sigma). Sample proteins were resolved by 2-DE using the 20 × 25 cm ISO-DALT™ 2D gel system (Hoefer Scientific Instruments, San Francisco, CA) [16]. Solubilized protein samples (175 μg) were applied to each isoelectric focusing (IEF) gel tube, and the gels were run for 25 000 Vh using progressively increasing voltage (500 V for 1 h, 750 V for 1 h, 1000 V for 1 h, and 1200 V for 19 h). A computer-controlled gradient casting system was used to prepare second-dimension SDS (sodium dodecyl sulfate) gradient slab gels in which the acrylamide concentration varied linearly from 11 to 17%T. First-dimensional IEF tube gels were loaded directly onto the slab gels. Second-dimensional slab gels were run in groups of 20 in a DALT slab electrophoresis tank at 10°C for 18 h at 160 V. Following SDS electrophoresis, slab gels were stained for protein using Coomassie Brilliant Blue G-250, a colloidal staining procedure, for 96 h, after which equilibrium intensity was achieved [17].

2.5 2-DE analysis

Stained gels were digitized at 125 micron resolution using a CCD (charge-coupled device) scanner and images were processed using the KEPLER™ software system to generate a parameter list giving *x, y* position, shape, and density information for each detected spot. Groupwise statistical comparisons were made to screen for protein alterations (two-tailed Student's *t*-test) and pattern uniformity (coefficient of variation, CV). Corresponding spot-integrated density information was presented graphically, as maps, bar graphs, and screen captures shown in Fig. 3–7. Consistency of protein spot abundance, *i.e.*, dispersion of values around group means, was accomplished by calculating the CV for each group and generating an average of all the individual CVs. CV was determined by dividing the standard deviation by the group mean and expressing this value as a percentage.

2.6 Immunoelectrophoresis

Protein patterns on replicate gels were electroblotted for immunological identification of glutathione-*S*-transferase (GST) protein spots. Proteins were transferred from the polyacrylamide gel slabs onto polyvinylidene difluoride (PVDF) membranes (in 49 mM Tris – 39 mM glycine buffer with 0.04% SDS and 20% methanol, pH 9.2) using a semidry transfer cell for 37.5 Vh at room temperature. The PVDF membranes were washed and blocked with several exchanges of 0.3% Tween-20 in phosphate buffered saline (PBS; 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.4) and incubated with primary antibody (1:2000; Biotrin, Dublin, Ireland) for 1.5 h. Blots were washed with 0.3% Tween-20 in PBS, incubated 1.5 h with an alkaline phosphatase-conjugated secondary antibody (Biotrin) and visualized using 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT, Sigma Fast™ tablets). Following visualization, all blots were rinsed with water and scanned as described below. They were then air-dried, blocked with 0.3% Tween-20 in PBS, rinsed with water, and stained with stabilized colloidal gold reagent [18].

2.7 Glutathione-*S*-transferase activity determination

GST activity was determined by assaying the amount of 1-chloro-2,4-dinitrobenzene (CDNB) conjugated with glutathione per mg of cytosolic protein [19]. CDNB was incubated with glutathione and a source of GST in phosphate buffer, pH 6.5. The change in absorbance at 340 nm was monitored for 2 min and GST activity was calculated as nmol/min/mg protein.

3 Results

3.1 Blood lead

A lead exposure of 5–8 weeks was necessary to achieve the target blood lead concentrations of 20, 40 and 80 μg/dL. Once these levels were obtained, rabbits were administered maintenance doses of lead. Blood lead levels fluctuated only slightly from targeted values for the remainder of the study (data not shown).

3.2 Pathology

Average body weight increased minimally, from 2161 to 2277 g, through the 20-week study irrespective of lead treatment (Fig. 1). There were no differences in the kidney/body weight ratio as the blood lead level increased (Fig. 2). Major organs, *i.e.*, the brain, liver, gall bladder, accessory sex organs as well as the kidney, were evaluated for pathological changes. No gross pathological

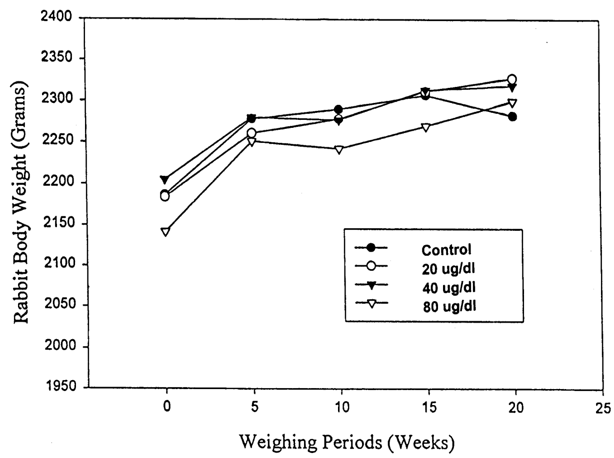

Figure 1. Mean rabbit weight *versus* weighing periods within each lead treatment group at 5-week intervals during study.

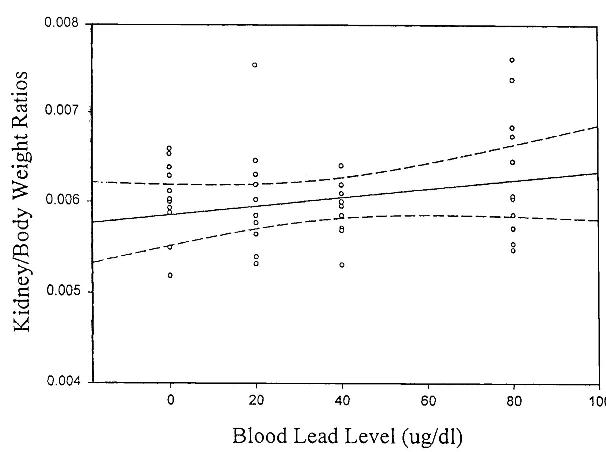

Figure 2. Correlation of kidney weight/body weight ratio to blood lead level. Dotted curve represents 95% confidence interval.

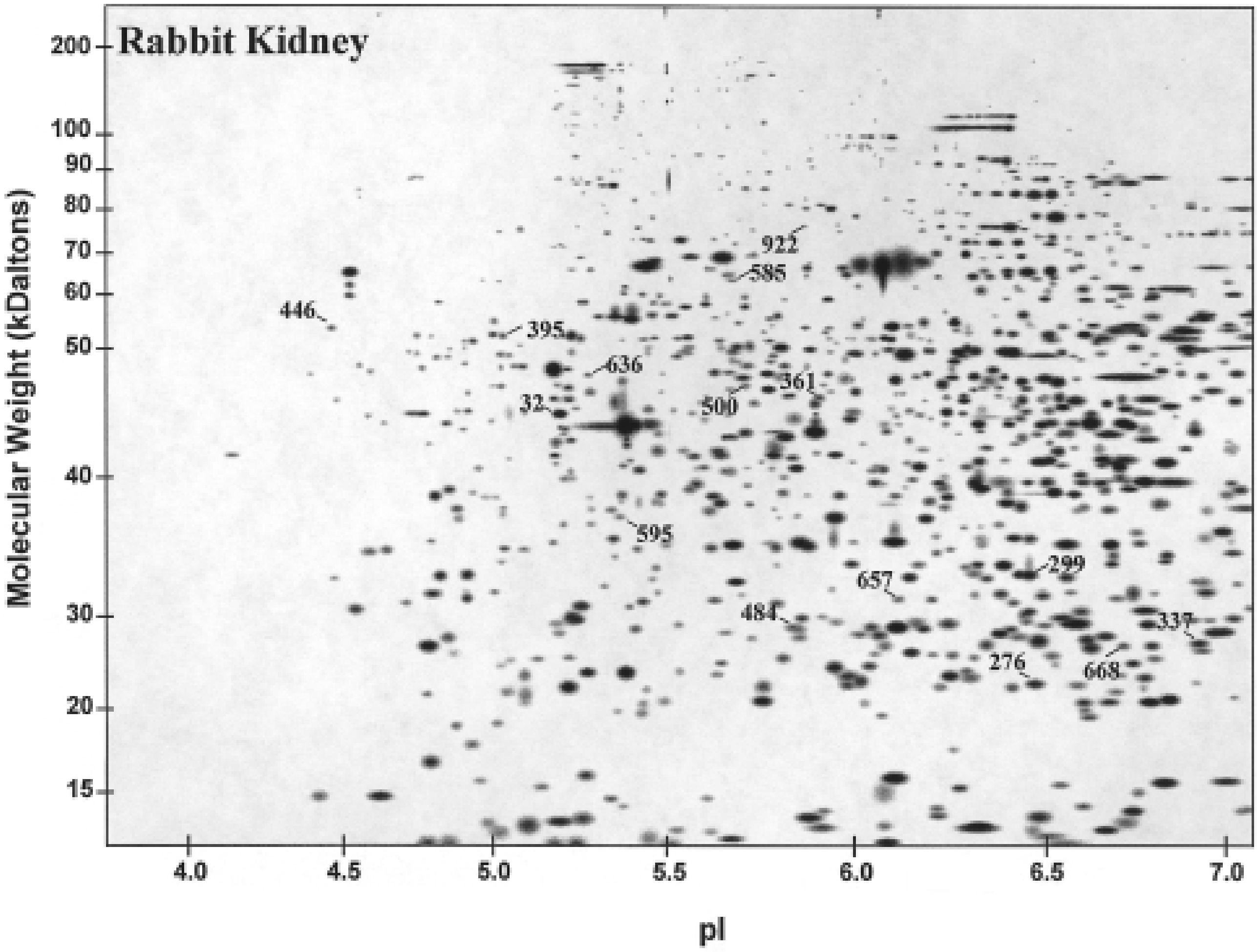

Figure 3. 2-DE protein master pattern of rabbit kidney. The proteins labeled by MSN are altered by lead exposure ($p < 0.005$). Acidic and basic proteins are oriented to the left and the right, respectively. Low M_r and high M_r proteins are oriented to the top and bottom, respectively. The gel pattern represents a vertical acrylamide gradient of approximately 11–17% and a horizontal pH gradient of approximately 4–7.5. Molecular weight and p*I* calibrations are estimates based on the calculated M_r and p*I* of protein spots from samples separated on replicate gels. This pattern includes all proteins detected in all samples and is thus a composite, not necessarily representative of any one sample pattern.

changes or histopathological lesions were noted in these organs at target blood lead levels, 20–80 μg/dL.

3.3 2-DE

A 2-DE master pattern of rabbit kidney, a composite of all protein spots resolved in the numerous sample patterns, is shown in Fig. 3. The average number of individual kidney proteins which could be reproducibly resolved and matched to the master pattern per sample, "matched spots" in Table 1, is 1100. There is a coefficient of variation of less than 15%, an indication of relative protein pattern consistency, for 200 (18%) of the matched spots (Table 1). The mean abundance of each matched protein spot in the master pattern was compared with that for the corresponding spot in the patterns from the lead-treated samples. Figure 3 displays the positions of those proteins identified by master spot number (MSN) that were significantly ($p < 0.005$) altered by lead exposure.

As an initial screen for renal markers of lead toxicity, a statistical analysis of protein abundance changes in the various sample patterns was performed. The protein alterations detected in the various exposure groups show a dose-related lead effect (Table 1). Only two protein spots were altered with a significance of $p < 0.001$, and these were in the highest lead level group (80 μg/dL). At $p < 0.005$, protein changes again occur only in the 80 μg/dL group with the exception of one protein which was altered in the 40 μg/dL level group, MSN 299. The dose-related nature of the renal effect is clearly demonstrated by the protein alterations compared by Student's *t*-test at

Table 1. Statistical analysis of kidney homogenates from lead-treated rabbits

	Number of protein spots (blood lead; μg/dL)	Percent matched protein spots
Matched protein spots[a)]	1100	–
CV < 15%	200	(18%)
$p < 0.001$ (*vs.* control)[b)]	2 (80 μg/dL)	(0.18%)
$p < 0.005$	1 (40 μg/dL)	(0.09%)
$p < 0.005$	14 (80 μg/dL)	(1.3%)
$p < 0.05$	12 (20 μg/dL)	(1.1%)
$p < 0.05$	25 (40 μg/dL)	(2.3%)
$p < 0.05$	102 (80 μg/dL)	(9.3%)

a) Average number of kidney protein spots in the master reference pattern (Fig. 1) reproducibly resolved and matched to the individual sample gel patterns in each group.
b) Student's *t*-test

a low significance level, $p < 0.05$. As Table 1 shows, the number of proteins exhibiting a change in mean quantity at this level increased almost exponentially with treatment. Alterations were noted in all lead groups, but the number increased with the level of lead (12, 25 and 102 proteins in 20, 40 and 80 μg/dL lead, respectively).

Individual abundances of the proteins identified by MSN in Fig. 3 are shown by bar graphs in Fig. 4. Bars are grouped by exposure and each individual bar represents the abundance of that protein in the kidney sample gel pattern. Most (11) of the fifteen protein alterations resulted from increased abundance. Figures 5 and 6 illustrate the modest changes in protein abundance associated with lead exposure. The expression of one of the induced proteins (twofold), MSN 446, a potential marker, is shown in Fig. 5. Figure 5, a screen capture of Kepler™ system montage format, illustrates that section of each gel pattern in which MSN 446 is resolved. The far-left frame in each row is the master pattern for that group. The remaining six frames show the sample pattern from individual gels. In contrast, the expression of a protein whose abundance declined significantly (approximately 50%), MSN 395, is shown in Fig. 6. The far-left frame illustrates the master pattern, and the remaining six frames show the sample pattern from individual gels within the corresponding treatment group.

Putative GST proteins were induced by lead exposure (Fig. 7) as was GST enzymatic activity, as measured by thioether formation (nmoles CDNB/min/mg). Four proteins cross-reacted with anti-rat GSTP1 (pi class GST Yp) antibody, MSN 187, 201, 246 and 392 (data not shown). The sum of the four spot abundances in each sample gives the total GSTP1 abundance. This figure parallels induced GST activity as determined in the same tissue samples (Fig. 8). In contrast, tentative identification of stress proteins (hsc 70, hsp 70 and hsp 90) based on homologous coordinate position as compared to kidney patterns from rodents revealed no change in abundance with lead exposure [20].

4 Discussion

Previous epidemiological evidence has shown that nephrotoxicity occurs in lead-exposed workers. In the present investigation we attempted to identify molecular correlates of renal toxicity in a rabbit model developed for assessing toxic effects of experimental lead exposures that mimicked occupational exposures. The rabbit provided an ideal experimental animal model for assessing the concentration-dependent effects of lead at blood levels below 100 μg/dL since the dosing procedure produced stable blood levels at or near the three targeted levels. Further, rabbit renal tissue exhibited a burden of lead that reflected the blood lead concentrations of the experimental groups. Choie and Richter [11] reported that rodent kidney responded to a single injection of lead (4 mg lead/100 g body weight) with a dose-dependent increase in body weight and kidney/body weight. In contrast, Kasprzak *et al.* [10] reported that feeding rats with a 1% lead diet for 18 months decreased body weight by 80% and increased the kidney/body weight ratio 200%. However, the response of rat and rabbit to lead appear to be quite different since in this investigation overt toxic effects of lead were absent. Following subchronic lead treatment in low to mid-range blood levels (20–80 μg/dL) body weight increased across all groups, and kidney weights were normal. Further, the kidney/body weight ratio showed only a slight increase with increasing lead exposure.

The histopathology of an acute lead renal exposure has been characterized by proximal tubular dysfunction and interstitial nephritis [21]. Acute toxic effects are primarily manifested as dilation of endoplasmic epithelium, bulbing of the nuclear membrane, changes in mitochondrial structure, and formation of inclusion bodies [22]. Chronic lead toxicity has been characterized as gradual tubular atrophy and interstitial fibrosis [23]. Chronic exposure can damage glomeruli and proximal tubules, affect glomerular filtration, renal clearance and tubular reabsorption, and eventually lead to renal failure [2]. In this study no renal

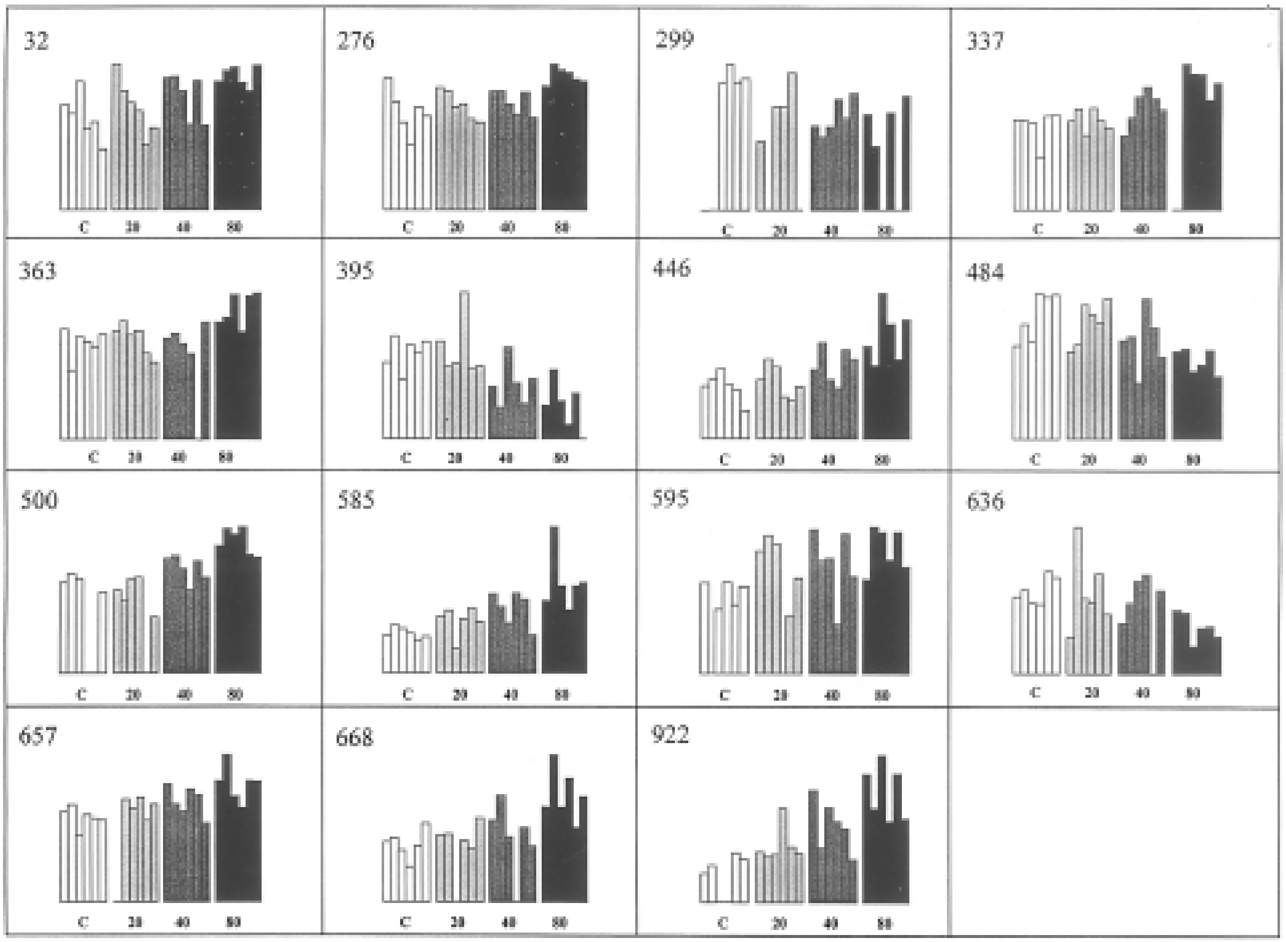

Figure 4. Bar graphs of rabbit kidney proteins significantly altered ($p < 0.005$) by lead exposure. Bars are grouped by exposure, and each individual bar represents the abundance of that protein (identified by MSN, see Fig. 3) in the kidney sample gel pattern. The abundances are integrated densities (product of optical density, *sx* and *sy*) and have no units. CV < 15%.

pathological lesions were noted at necroscopy, although it is possible that any lesions involved only highly subtle renal tubular degeneration. Interestingly, in rabbits which received higher doses of lead (over 100 µg/dL), tubules at the cortico-medullary junction were slightly dilated and lined by squamous epithelial rather than cuboidal cells. Further, nuclei of the epithelial cells contained eosinophilic intranuclear inclusion bodies.

Lead is a powerful enzyme inactivator and reduces oxidative phosphorylation in kidney mitochondria [24]. It impairs cation transmembrane transportation in mitochondrion through inhibition of sodium and potassium ATPases [25]. It has also been reported that lead acetate is a strong protein synthesis inhibitor in rabbit reticulocytes *in vitro* [26]. Choie and Richter [11] reported that a single injection of lead to rats (4 mg/100 g body weight) resulted in an increased incorporation of labeled protein and nucleic acid precursors *in vivo* accompanied by an increased proliferation of proximal tubular epithelium over a period of 72 h. Another investigation by Choie and Richter [22] revealed an increase in protein synthesis following acute lead administration at a low level (0.54 µg/100 g body weight). In this study 2-DE was employed to detect dose-dependent changes in gel protein profiles obtained from lead-exposed rabbit kidney. Our study of protein profiles revealed that significant quantitative variation occurred in a small subset of over 1100 matched proteins. The Student *t*-test at $p < 0.005$ revealed that protein alterations occur only in the highest treatment group, 80 µg/dL, with the exception of one protein, MSN 299. However, at $p < 0.05$ the protein alterations detected in the various lead exposure groups clearly showed a dose-related renal effect. At this significant level the number of protein quantity changes increased almost exponentially with treatment (Table 1).

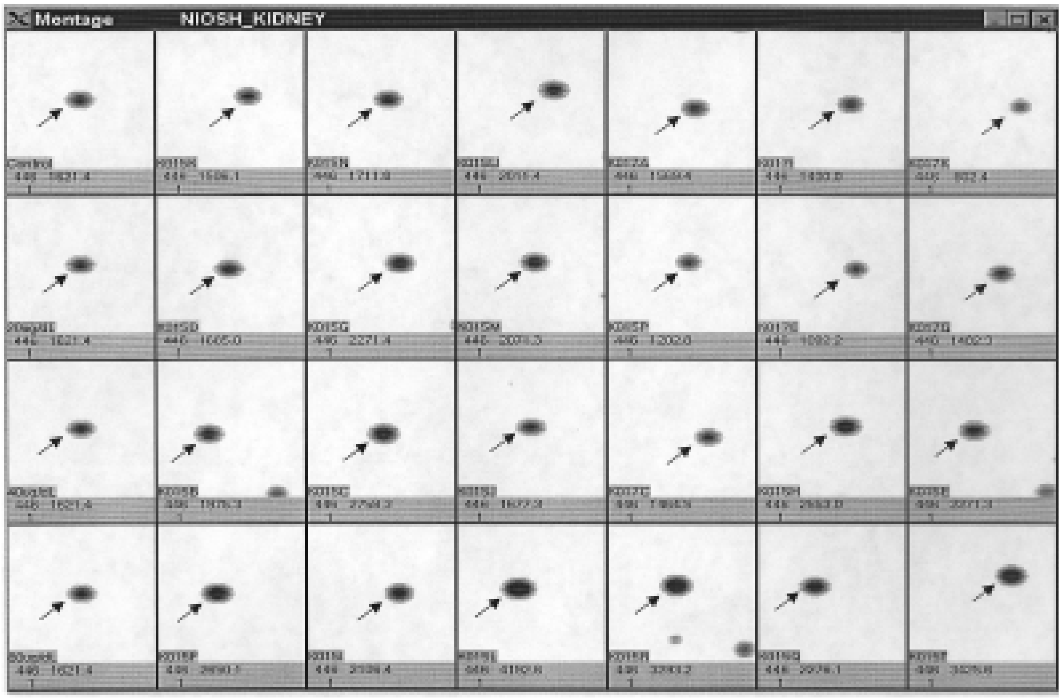

Figure 5. The effect of lead exposure on MSN 446 abundance. The first montage frame in each row represents the reference or master pattern. The top row shows that portion of each sample containing MSN 446. The second row shows the 20 µg/dL exposure, the third row 40 µg/dL, and the fourth row 80 µg/dL.

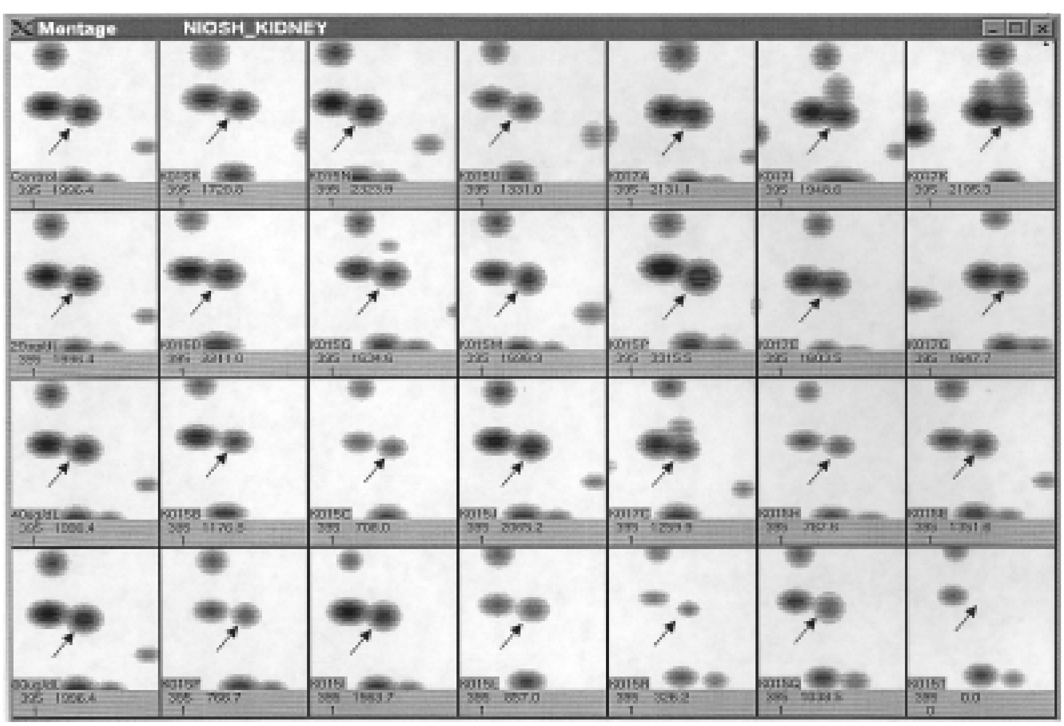

Figure 6. The effect of lead exposure on MSN 395 (arrows) abundance. The first montage frame in each row represents the reference or master pattern. The top row shows that portion of each sample containing MSN 395. The second row shows the 20 µg/dL lead exposure, the third row 40 µg/dL, and the fourth 80 µg/dL.

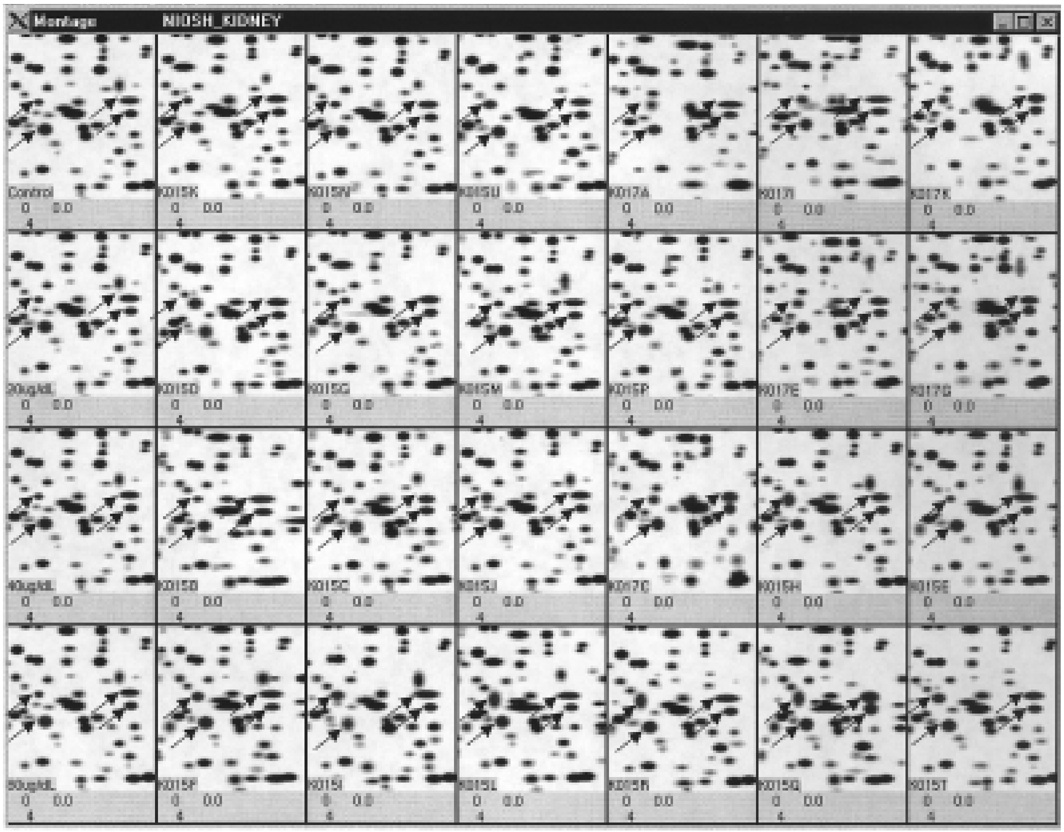

Figure 7. The effect of lead exposure on MSN 187, 201, 246 and 392 (arrows) abundance. The first montage frame in each row represents the reference or master pattern. The top row shows that portion of each sample containing MSN 187, 201, 246 and 392. The second row shows the 20 µg/dL lead exposure, the third row 40 µg/dL, and the fourth 80 µg/dL.

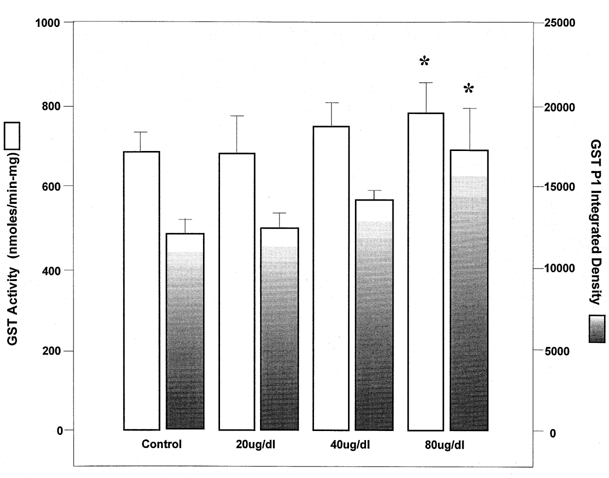

Figure 8. The effect of lead exposure on total GST activity (measured by thioether formation) and total putative GSTP1 abundance (sum of integrated densities of MSN 187, 201, 246 and 392). Values are means ± SEM. * $p < 0.05$ following ANOVA (analysis of variance) *via post hoc* multiple range analysis.

The blood lead levels studied have clearly altered the normal pattern of kidney protein expression. These observations are without precedent. Protein databases of kidneys from experimental animals are not numerous. Previously, we have observed severe acute effects in Sprague-Dawley rats subjected to short-term, high lead exposure (114 mg/kg, three intraperitoneal injections daily). Here 36% of matched proteins in the cytosolic fraction prepared from whole kidney were altered at $p < 0.05$ and 17% at $p < 0.005$ [27]. To our knowledge, however, the present study represents the first presentation of a 2-DE protein expression profile of rabbit kidney. Additionally, the relatively low blood levels used in this study are comparable to those seen in the exposed worker. This is significant since this model may thus provide an enhanced understanding of the biochemical mechanism for lead-induced toxicity.

The physiological significance of the observed protein alterations in kidney cannot be accurately assessed until the altered proteins are identified. Of particular interest are MSN 446 (Fig. 5), which demonstrated a mean dose-related increase in abundance, and MSN 395 along with the possible charge variant of MSN 395 (Fig. 6), which demonstrated a dose-related decrease. Additionally, MSN 187, 201, 246 and 392 were induced to a lesser extent (Fig. 7). Based on their coordinate positions and immunoreactivity to rat anti-GSTP1 (glutathione-*S*-transferase), these proteins may be GSTP1 charge variants (Fig. 8). This identification and induction corresponded with previous rodent studies in our lab which identified the various GST charge variants and documented lead-mediated inductions and charge modifications in Yp and Yb GST [27].

Our experiment has begun to reveal the kidney protein profile of the lead-exposed rabbit. We have exposed these to relatively low blood levels, 20 and 40 µg/dL, which are representative of that seen in the workplace. Further development of this *in vivo* model, by conclusive protein identification and expansion of the pH gradient using first-dimensional IPG separation, is planned. These studies will provide enhanced understanding at the biochemical level of the lead-induced toxic effect on the kidney and offer additional insight into the putative basis for lead nephrotoxicity in exposed workers.

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5 References

[1] National Institute for Occupational Safety and Health, *National Occupational Exposure Survey*, US Department of Health and Human Services, Cincinnati, OH 1983.

[2] Nolan, C. V., Shaikh, Z. A., *Toxicology* 1992, *73*, 127–146.

[3] Vostal, J., Heller, J., *Environ. Res.* 1968, *2*, 1–10.

[4] Vander, A. J., Taylor, D. L., Kalitis, K., Mouw, D. R., Victery, W., *Am. J. Physiol.* 1977, *233*, 532–538.

[5] Victery, W., Vander, A. J., Mouw, D. R., *Am. J. Physiol.* 1979, *237*, 398–407.

[6] Victery, W., Vander, A. J., Mouw, D. R., *Am. J. Physiol.* 1979, *237*, 408–414.

[7] Goyer, R. A., *Curr. Topics Pathol.* 1971, *55*, 147–176.

[8] Wedeen, R. P., Maesaka, J. K., Weiner, B., Lipat, G. A., Lyons, M. M., Vitale, L. F., Joselow, M. M., *Am. J. Med.* 1975, *59*, 630–641.

[9] Kim, R., Rotnisky, A., Sparrow, D., Weiss, S., Wager, C., Hu, H., *JAMA* 1996, *275*, 1175–1181.

[10] Kasprzak, K. S., Hoover, K. L., Poirer, L. A., *Carcinogenesis* 1985, *6*, 279–282.

[11] Choie, D. D., Richter, G. W., *Am. J. Pathol.* 1972, *66*, 265–275.

[12] Wibow, A. A., Zielhuis, R. L., *Arch. Toxicol.* 1980, *45*, 67–73.

[13] Spit, B. J., Wibowo, A. A., Feron, V. J., Zielhuis, R. L., *Arch. Toxicol.* 1981, *49*, 85–91.

[14] Moorman, W. J., Skaggs, S. R., Clark, J. C., Turner, T. W., Sharpnack, D. D., Murrell, J. A., Simon, S. D., Chapin, R. E., Schrader, S. M., *Repro. Toxicol.* 1998, *12*, 333–346.

[15] Toraason, M., Moorman, W., Mathias, P., Fultz, C., Witzmann, F., *Electrophoresis* 1997, *18*, 2978–2982.

[16] Witzmann, F. A., Fultz, C. D., Mangipudy, R. S., Mehendale, H. M., *Fundam. Appl. Toxicol.* 1996, *31*, 124–132.

[17] Neuhoff, V., Arold, N., Taube, D., Ehrhardt, W., *Electrophoresis* 1988, *9*, 255–262.

[18] Fultz, C. D., Witzmann, F. A., *Anal. Biochem.* 1997, *251*, 288–291.

[19] Habig, W. H., Jakoby, W. B., *Methods Enzymol.* 1981, *77*, 398–405.

[20] Witzmann, F. A., Fultz, C. D., Grant, R. A., Wright, L. S., Kornguth, S. E., Siegel, F. L., *Electrophoresis* 1999, *20*, 943–951.

[21] Goyer, R. A., *Am. J. Pathol.* 1971, *64*, 167–182.

[22] Choie, D. D., Richter, G. W., *Effects of Lead on the Kidney*, Urban and Schwarzenberg, Baltimore 1980, pp. 187–195.

[23] Goyer, R. A., *Toxicol. Lett.* 1989, *46*, 153–162.

[24] Lockitch, G., Clinical Biochem. 1993, *26*, 371–381.

[25] Goyer, R. A., *Environ. Health Perspect.* 1990, *86*, 177–181.

[26] Borsook, H., Fischer, E., Keighley, G., *J. Biol. Chem.* 1957, *229*, 1059–1070.

[27] Witzmann, F. A., Daggett, D. A., Fultz, C. D., Nelson, N. A., Wright, L. S., Kornguth, S. E., Siegel, F. L., *Electrophoresis* 1998, *19*, 1332–1335.