Korean J. of Toxicology
Vol.1, No.1, p49-63, 1985

STUDIES OF THIAMIN (VITAMIN B_1) EFFECTS ON LEAD POISONING IN RATS

Jong-Koo Kang, Joon-Sup Lee, Yong-Soon Lee
College of Veterinary Medicine, Seoul National University
Suwon City, Kyungki-do 170, Korea

= *ABSTRACT* =

The purpose of this study was to investigate whether or not Vitamin B_1 (thiamin hydrochloride) could prevent and/or treat lead poisoning in rats. Sprague-Dawley used in this experiment were divided into three group; control group compound group (thiamin 10mg+0.4% lead acetate solution) lead group (0.4% lead acetate solution).

The results obtained were summarized as follow;

1. *The body weight gain of treated groups (compound group and lead group) were lowered significantly in comparison with those of control groups ($p<0.01$). But the significant difference between treated group was not observed ($p>0.05$).*
2. *The weight ratios of each organ to body weight of treated groups were increased significantly in comparison with those of control groups ($p<0.01$). But the significant difference between treated group was not observed ($p>0.05$).*
3. *All of the treated groups were lowered significantly in comparison with control group in all the parameters (Rbc, Hb, Hct, MCV, MCH) except for reticulocyte number ($p<0.01$). In reticulocyte number, at 3 weeks lead group was increased significantly in comparison with other groups($p<0.01$). At 6 weeks reticulocyte number of lead group lowered as control group.*
4. *Compound group were changed slightly in comparison with lead group in histological changes of kidney and liver; hypertrophies of nuclei, cell necrosis, increase of size and number of intranuclear inclusion bodies.*

Key words: *Lead poisoning, Rat, Thiamin hydrochloride, Lead acetate, Intranuclear inclusion body, Reticulocyte,*

INTRODUCTION

It is well known that leads have occurred to disturbance of central nerve system (Lefauconnier et al., 1973), peripheral nerve system (Hunter and Wobester, 1979), kidney (Chisolm, 1962), hematopoietic system (Stofen, 1974) and reproductive system (Kennedy et al., 1971). It has been reported that leads are more susceptible to the children than to the adults (Ziegler et al., 1978), are more susceptible to the male than the female in rat (Kostial et al., 1971) and there are strain differ-

ences in lead intoxication (Mykkanen et al., 1980).

As the pathological findings of lead poisoning, necrosis of proximal tuble, interstitial nephritis, hypertrophy of nucleus, hypertrophy of cell, formation of inclusion body and so on have been known (Jones and Hunt, 1983). The formation of inclusion body would considered to be pathognomonic sign of lead poisoning, it has been reported in hepatocyte and proximal tubular cell by Blackman (1936), osteoclast cells (Hsu et al., 1973), renal epithelial cells in tissue culture (Walton, 1974), some endothelial cell in the brain (Clasen et al., 1974) and Schwann cell (Powell et al., 1982).

Lead were mainly absorbed at duodenum (Conrad and Braton, 1978), accumulation amounts were said to be the process of bone, kidney, liver, brain (Keller and Doherty, 1980).

In many reports about correlation of lead and other materials, it have been found that iron (Six and Goyer, 1972), calcium (Rosen, 1983), phosphorus (Aungst and Fung, 1983), copper (Klauder and Petering, 1977), zinc (Cerklewski, 1984), protein (Mylroi et al., 1977) and tin (Vander et al., 1979) decrease the retention of lead and relieve the lead intoxicaton. However, lactic acid (Bushneli and DeLuca, 1981), fat (DeLuca et al., 1982), citric acid (Furia, 1968) and so on increase the absorption and accumulation of lead. But it is not evident yet why and how corelation between lead and these materials occur.

In lead intoxication as reports about the effects of vitamins, these facts suggest that nicotinic acid (Silvestroni et al., 1965), vitamin B_6 (Pokotilenko, 1964), vitamin B_{12} (Garminati, 1959), ascorbic acid (Pillmer, 1940) and so on decrease slightly the toxicity of lead but vitamin D (Smith et al., 1978) increase the lead toxicity.

Bratton et al. (1981a, b,) have reported that although lead groups are died by severe poisoning signs in ruminant but in compund groups clinical signs disappear, no death occur and lead levels in soft tissue remain below the confirmatory level associated with lead poisoning. However, no studies about correlation of lead and thiamin have been reported. The purpose in the present sutdy was carried to investigate whether or not thiamin could prevent and/or treat lead poisoning in rats.

METHODS AND MATERIALS

1. Experimental animal

60 to 80 days old Sprague-Dawley male rats of 75 rats (Body weight 135 to 155g) were obtained from the Laboratory Animal Breeding Center, Seoul National University. They had access to food and water ad libitum and were housed at room temperature under natural light.

Laboratory animals were equally divided into 3 groups of control group, lead group, compound group. Each group consisted of 24 rats. Five rats from each group were killed at 1 week, 2 weeks, 4 weeks, 6 weeks, over 6 weeks period after lead exposure. In control group, 0.5cc saline was administered daily by SC and distilled water as drinking water was provided. In lead group, o.5cc saline was administered daily by SC and 0.4% lead acetate (Hanawa Co.) solution as drinking water was provided. In control group, 10mg thiamin hydrochloride (Vitamin B_1, Junsei Co.) in 0.5cc saline was administered by SC and 0.4% lead acetate solution as drinking water.

2. The measurement of body weight and each organ weight and the method of blood collection

Body weights were measured before sacrifice at 1 week, 2 weeks, 3 weeks, 4 weeks, 6weeks. Animals were anesthetized with sodium pentobarbital (Pitman-Moore Co.) by IP and blood was collected by heart puncture. After blood collection, the animals were decapitated and the kidney, liver, spleen, brain and testis were excised and weighed. The results of weight measurement were analyzed statistically using Student test.

3. Hematological observation

In order to obtain reticulocyte counts, blood was mixed with 0.5% new methylene blue as the same ratio (1:1). After 10 minutes, samples were smeared, counterstained. with Wright solution. At least 1,000 red blood cell (RBC) were counted on slide from each rat. On the other hand red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) were made manually with Coulter counter (Model S-plus Coulter electronic Co.)

4. Microscopical finding

After gross finding of kidney and liver, the samples were fixed in buffered neutral 10% formalin, embedded in paraffin, sectioned to 5 μm and stained with Hematoxylin-Eosin

5. Electron microscopical finding

After out-bleeding, kidney and liver were sliced to 1mm thickness and samples were fixed with 5% glutaraldehyde in Sorensen's buffer (PH 7.2) and postfixed with 1% osmic acid in Sorensen's buffer (PH 7.2). After dehydration with acetone and alcohol, all of the speicmens were embedded in Epon mixture (Epon 812, DDSA, MNA, DMP 30), sectioned to lum with ultramicrotome (Sorvall-MT 5,000). Thick sections were stained with toluidine blue for light microscopical observation. After observation of the incidence of inclusion bodies, the sample was sectioned by 60 to 90mm, double-stained with lead citrate and uranyl acetate, examined in transmission electron microscope (JEOL-120 CX II)

RESULT

1. The results of weight measurement

The results of body weight measurement increased in proportion to the time passage as shown in Fig. 1 and at 6 weeks all of the groups had a significant difference.

The weight of lead group and compound group were lowered significantly in comparision with control group ($p<0.01$). But difference between lead group were very small. The weight ratios of each organ to body weight have partly significance along the time passage as shown in Table 1. It's ratio of treated groups were increased significantly in comparison with control groups during experimental periods ($p<0.01$). However the significant difference between treated group was not observed in all organ ($p>0.05$).

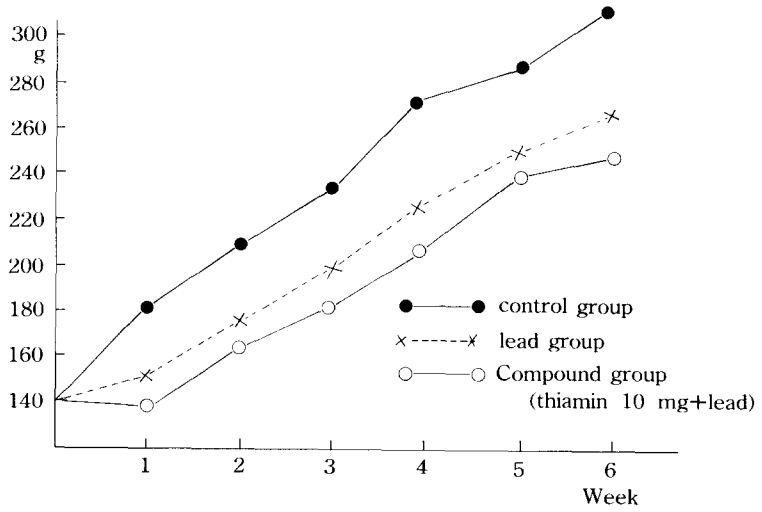


Fig. 1. Comparison of body weights of each group.

Table 1. Comparisions of each organic weight (% of body weight) of each group.

Group \ Period \ Item		Kidney weight ratio	Liver weight ratio	Spleen weight ratio	Brain weight ratio	Testis weight ratio
Control	1 week	0.87±0.07	3.78±0.40	0.24±0.07	0.83±0.06	1.33±0.10
Lead G		1.06±0.09	3.96±0.43	0.30±0.07	0.97±0.10	1.36±0.09
Compound G		0.95±0.07	3.74±0.58	0.30±0.08	0.98±0.09	1.34±0.09
Control	2 week	0.78±0.02	3.67±0.49	0.21±0.03	0.78±0.04	1.17±0.12
Lead G		0.88±0.06	3.97±0.40	0.32±0.02	0.87±0.03	1.39±0.15
Compound G		0.88±0.03	3.94±0.18	0.28±0.04	0.85±0.04	1.27±0.14
Control	3 week	0.73±0.12	3.48±0.48	0.22±0.03	0.65±0.08	1.09±0.12
Lead G		1.02±0.14	3.92±0.34	0.28±0.07	0.77±0.03	1.32±0.15
Compound G		0.98±0.16	3.74±0.18	0.34±0.04	0.80±0.09	1.34±0.09
Control	4 week	0.69±1.33	3.71±0.26	0.19±0.03	0.58±0.05	0.95±0.10
Lead G		0.94±0.07	3.70±0.31	0.28±0.04	0.71±0.06	1.23±0.16
Compound G		0.78±0.06	3.59±0.24	0.24±0.05	0.70±0.07	1.17±0.07
Control	5 week	0.73±0.03	3.35±0.22	0.19±0.02	0.49±0.02	0.89±0.07
Lead G		0.94±0.10	3.35±0.38	0.21±0.38	0.60±0.05	1.13±0.09
Compound G		0.95±0.04	3.60±0.33	0.21±0.03	0.60±0.05	1.05±0.09

Mean±S.D.

2. The results of hematological observation

The results of hematological observation were as shown Table. 2.

The treated groups were lowered significantly in comparison with control group in all the parameter (Rbc, Hb, Hct MCV, MCH,) ($p<0.01$). However the significant difference between treated groups were not observed.

In reticulocyte number control groups were not nearly changed, but treated group were highered significantly in comparison with control group during all the

Table-2 Comparison of Hematological changes of each group

Group \ Period \ Item		RBC (10^6/mm^2)	Hct (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	Reticulocyte No.
Control group		8.8±0.4	49.6±1.1	15.4±0.4	57.0±1.3	17.5±0.3	25.8±7.5
Lead	1 week	8.6±0.2	45.4±0.4	13.5±0.3	54.6±3.3	16.3±0.6	56.8±5.7
Compound	1 week	8.2±0.4	45.0±1.5	13.8±0.9	54.8±4.1	16.8±0.7	32.2±9.5
Control group		8.7±0.7	49.2±3.4	15.0±0.7	57.2±2.1	17.2±0.5	24.8±6.4
Lead	2 week	8.2±0.7	44.7±2.1	13.2±0.6	54.5±1.6	16.2±0.4	82.8±13.0
Compound	2 week	8.3±0.4	43.3±1.8	13.7±0.6	52.1±1.2	16.5±0.6	48.3±4.5
Control group		8.8±0.2	50.3±1.9	14.9±0.8	57.1±1.5	16.9±0.4	22.0±4.9
Lead	3 week	8.1±0.6	44.0±1.2	12.5±0.5	54.3±1.8	15.4±0.3	100.0±21.3
Compound	3 week	8.2±0.3	42.8±1.1	12.3±0.5	52.1±1.8	15.2±0.4	81.0±16.5
Control group		8.5±0.7	49.0±2.1	15.3±0.8	56.3±1.9	17.5±0.3	18.2±3.8
Lead	4 week	7.8±0.3	43.2±1.5	12.5±0.2	52.0±2.2	15.0±0.5	62.0±5.7
Compound	4 week	8.2±0.4	41.9±1.8	12.0±0.6	51.1±1.2	14.6±0.5	50.6±15.0
Control group		8.6±0.5	49.8±2.3	15.1±0.8	57.8±1.6	17.3±0.6	15.2±5.7
Lead	6 week	8.2±0.3	42.4±1.2	12.4±0.2	50.6±1.2	15.0±0.3	20.2±11.0
Compound	6 week	8.1±0.3	41.0±1.9	12.3±0.5	50.4±1.8	15.1±0.5	24.4±10.6

times except for 6 weeks. But at 6 weeks the number of lead group had fallen to number similar to those observed in control group. In morphology of Rbc at 1 week reticulocytes and leptocytes are observed in treated group (Fig 2). At 2 weeks the increase of reticulocyte were processed but morphological change of Rbc did not occur. At 3 to 4 weeks many reticulocyte were observed (Fig 3). At 6 week morphology of Rbc were shown to stomatocyte, target cell, anisocytosis, poikilocytosis (Fig 4).

3. Histopathological finding

Macroscopical appearance of kidney were appeared as slightly edematous characters and the increase of it's size was observed at treated groups.

In microscopical finding the slight hypertrophy of proximal tubules and the bleeding of cortex sites started in lead group were severely changed than compound group at 4 weeks : epithelial cell of low portion of cortex were irregularly observed to various forms and size and also hyaline concretion and cell debris with large nucleus was occasionally observed in lumen (Fig. 5,6). These epithelial cell had severe regenerative change and the cytoplasm were irregularly stained and also severe nephrosis were observed in medullary tubules (Fig. 7). However the process of these lesions was slow in compound group (Fig. 8). At 6 weeks, sloughing cells and hypertrophied nuclei were observed with severe tubular necrosis. These proximal tubular cells were without or with brush borders. Hypertrophy of interstice and fibrosis were observed at low portion of cortex and so tubular cell had lost their characteristic appearance (Fig. 9).

These intranuclear inclusion bodies were observed in H-E section by eosinophilic mass with the slight margination of chromatin but these could be suspected (Fig. 10). In toluidine blue stain, numerous lysosomes and inclusion bodies appeared in proximal tubular lining cells (Fig. 11). The size and number of inclusion bodies in one nucleus was various and its color was dark blue. At 3 weeks, inclusion bodies were rare and at 4 weeks occasionally observed in nucleus of proximal tubules and the size and number of inclusion bodies were increased along the time passage (Fig. 12). Specially, the size and number of inclusion bodies of compound group have decreased in comparison with lead group (Fig. 13). In election microscopic finding (Fig. 14, 15.) the central portions of inclusion bodies were denser than the peripheral portions of them and the latter were hair like shape.

In macroscopical finding of liver, the differences between treated group and lead group were not observed. In microscopical finding, central cells of hepatic lobule of treated group appered to be hypertrophic. The swelling of sinusoid and the binucleated cells were variously observed but cell necrosis not observed by 3 weeks (Fig. 16). At 4 weeks occasionally inclusion bodies were appeared in hypertrophied nucleli but the regenerative changes of hepatocytes were less than kidney cell and many hepatocytes with small vacuoles were observed (Fig. 17). At 6 weeks number of inclusion bodies in treated group increased, especially in lead group their characterstic appearance was disappeared by severe hepatocyte necrosis and the sizes of vacuoles were more increased.

DISCUSSION

The ratios of body weight gain were the basis to the metabolic effect of lead and decreased along the increase of lead injection. However, the relationship of lead & thiamin was not yet reported. As for the single effect of thiamin, Richards (1945) suggested that excess thiamin administered parenterally reduced the body weight in young rats. In this experiment, lead treated groups were significantly lowered than control groups and the changes of body weight gain were similar to the report of Murakami et al. (1983) but lead groups were slightly heavier than compound groups. Although the weight of compound groups was not due to the toxicity of excessive thiamin.

It has been reported that renal tubular dysfunction is shown to excess aminoaciduria, glycosuria, hyperphosphaturia (Chisolm, 1962) and in acute lead poisoning the cell proliferation of renal tubular cells was said to owing to a stimulation in the biosynthesis of DNA and RNA (Choie and Richter, 1972 a). It suggested that the weight increase of kidney are not the water increase in the tissue but was the increase of protein and nucleic acid. (Hirsch, 1973). In this experiment the kidney weight was reached at maximum before and after 3 weeks and these results were earlier than that of Murakami et al. (1983). This time difference was due to that although Murakami et al. (1983) used adult rat weighing 360.0±5.9g but author used young rat weighing 141.0±7.0g.

The weight ratio of liver to body weight was decreased along the age (Rikans, 1984). Choiodi and Cardeza (1949) have reported that compound group fed on a high fat containing lead acetate were more severe than general group in liver damage and anemia. Columbano et al. (1983) have observed the weight increase by hypertrophy of liver during intravenous injection of lead nitrate. Although weight gain of liver was not to be a certain symptom of lead poisoing, the lead groups were

a considerable weight gain during experiment period but at 6 weeks suddenly depressed. These results were consistent with Hirschs (1973)' observation and due to atrophy of hepatocyte and fatty changes. Especially compound groups were not suddenly depressed in comparison with lead groups and so it considered as thiamin may prevent the atrophy and fatty degeneration by lead poisoning.

Michaelson (1973) suggested that brain was a sensitive organ to lead toxicity and the weight increase of brain was due to the increase of water by edema. Bratton et al. (1981, a.b) have reported that thiamin decreased significantly the deposition of lead in both the central and peripheral nerve system and thus prevented clinical sign and death. However this experiment result recognized the effect of thiamin against lead poisoning.

Stow et al. (1973) have observed that the seminiferous tubules of lead-fed dogs contained many spermatogonia with greatly distended cytoplasm indicative of hydropic degeneration but their weights were not increased. In the result of these studies, compound groups have been continuosly appeared a lower weight ratio than lead groups, thus our data suggested that thiamin may prevent the damage of testis in lead poisoning.

Miller et al. (1983) have suggested that when lead was absorbed across the gut, lead interfered with the transport of other iron, especially reduction in availability of essential trace element such as zinc and iron may be responsible for the decrease in spleen weight. A true dependent decrease of spleen was observed during experiments period and these results were consistent with the report of Miller et al. (1983). At 1 week spleen had a considerable weight gain in treated groups than in control groups. At 6 weeks it's decrease was suddenly occurred. Because the significance between compound group and lead group were not observed, thiamin was considered as not having a certain effect to spleen.

In lead poisoning, it was reported that microcytic hypochromic anemia occurred (Klauder and Petering, 1977). In hematological results there were not significant difference between compound group and lead group and these results were the same that Bratton et al. (1981 b) observed in cattles. The reticulocyte numbers of compound group were lowered than those of lead group and these results were thought to the evidence which anemic signs were not severe. But owing to the destruction of bone marrow in reticulocyte number, all treated groups were almost near to normal at 6 weeks. According to these results, thiamin was considered as having a certain effect to hematopoietic system.

Many findings observed in these studies were faster than those of Murakami et al. (1983) in the process of lesion and moreover at 3 weeks inclusion bodies were occasionally observed. These results were considered as owing to the age difference. Angevine et al. (1962) said that inclusion bodies in renal epithelial cell was the characteristics of chronic intoxication. Richter (1976) have reported that inclusion bodies were produced within 24 hours in renal epithelial cell of rats and mice by injecting a single dose of lead acetate either intraperitoneally (100μPb/g) or into the heart (10μPb/g). Choie and Richter (1972 b) have observed that inclusion bodies were produced within 6 hours after intracardiac injection and so inclusion bodies only were not the characteristics of chronic intoxication. McLachlin et al. (1980) have observed that inclusion bodies were detected in the cytoplasm within 4 hours and in the nucleus at 24 hours. Walton (1974) have reported inclusion bodies in the nucleus at 1 hour after exposure of kidney tubule cell cultures to lead in sucrose solution.

Especially Goyer et al. (1970 b) have suggested that 60 to 100 times than the organ in which they found, approximately 80 to 90% of reanl lead is concentrated within the nucleus and at least 50% of the nuclear lead may be recovered as inclusion bodies. Goyer et al. (1979 a) have suggested that lead accumulates in the intranuclear inclusion bodies, thereby sparing toxic injury to cytiplasmic organelles and also lead-induced inclusion bodies are composed of lead and protein probably in the form of a lead protein complex which is not easily to be soluble. Dubenkemp (1984) have reported that the nuclear volume increase cannot be considered the formation of space-requiring inclusion bodies or pathological swelling because it began long before the first appearance of inclusion bodies and is regarded as the expression of active self-protecting effort of the cell and not as the sign of lead-induced damage.

In these experiments nuclear hypertrophy began to appear at 2 weeks and inclusion bodies were easily observed in hypertrophied nucleus at 4 weeks. These findings were consistent with the report of Duvenkemp (1984). Especially compound groups were slighter than lead group in the hypertrophy of cell and less than lead group in number of inclusion bodies and so thiamin was considered as having the effect which prevented lead accumulation in kidney than any other organs. In electron microscopical finding the form of inclusion bodies were consistent with the observation of Richter et al. (1969) and Beaver (1961) and the increase of inclusion bodies was observed to occur form peripheral sites along the time passage.

It has been reported that lead results in fatty infiltration or degeneration, hypertrophy of nucleus, necrosis of hepatocytes, fibrosis with lobular atrophy and cirrhosis, among other. Blackman (1936) found inclusion bodies in the liver cells, many abnormal nuclei with or without inclusion bodies and occasional necrotic cells. Columbano et al. (1980) observed that the number increase of hepatocyte occurred but cell necrosis did not occur. In this experiment the change of parenchymal cell was hard to observe but mitosis was often observed and these lesions were consistent with the report of Chiodi and Cardeza (1949). During the experimental period compound groups were changed slightly in comparision with lead groups in fatty degeneration, vacuolization, formation of inclusion bodies. As the results of studies although the action site and mechanism of thiamin has not yet been explained, thiamin has been considered to be a protective action in the liver also.

On the other hand, the artificial chelating agents used in treatment of lead poisoning have been Ca-EDTA, BAL, D-Penicillamine. Owing to the side effects these chelators could be by means totally effective. But It was assumed that because excess parentally administered thiamin was fastly excreted in urine and had not the toxicity and so was effective to the treatment and prevent (Bratton et al. 1981. a.b.).

Our data suggested that although injection dose of thiamin was approximately 20 times the required daily intake (4mg/kg body weight), nevertheless the thiamin own toxcity were not observed, especially compound groups were milder than in comparision with lead groups in the histological changes of kidney and liver, the increase of size and number of intranuclear inclusion bodies, the changes of hematopoietic funcition and as a result of these studies thiamin was effective to the prevention and treatment of lead poisoning.

REFERENCES

Angevine J M, Kappas A, DeGowin R L and Spargo B H (1962) Renal tubular nuclear inciusions of lead poisoning. *Arch. Pathol.*, 72:486-494.

Aungst B J and Fung H-L (1983) Inhibition of oral lead absorption in rats by phosphate containing products. *J. Pharmaceut. Sci.*, 72:345-348.

Beaver D L (1961) The ultrastructure of the kidney in lead intoxication with particular reference to intranuclear inclusions *Am. J. Pathol.*, 39:195-200.

Blackman S .S (1936) Intranuclear inclusion bodies in the kidney and liver caused by lead poisoning. *Bull. Johns Hopkins Hosp.*, 58:384-404.

Bratton G R, Zmudzki J, Bell, M C and Warnock L G (1981 a) Thiamin (Vitamin B) effects on lead intoxication and deposition of lead in tissue : Therapeutic potential. *Toxicol. Appl. Pharmacol.*, 59:164-172.

Bratton G R, Zmudzki J, Kincaid N and Joice C (1981 b) Thiamin as treatment of lead poisoning in Ruminant. *Mod. Vet. Pract.*, 62:441-446.

Bushneli P J and DeLuca M F (1981) Lactose facilitates the intestinal absorption of lead in weanling rats. *Science*, 211: 61-63.

Cerklewski F L (1984) Postabsorptive effect of increased dietary zinc on toxicity and removal of tissue lead in rats. *J. Nutr.* 550-554.

Chiodi H and Cardeza A F (1949) Hepatic lesions produced by lead in rats fed a high fat diet. *Arch. Pathol.*, 48:395-403.

Chisolm J J (1962) Aminoaciduria as a manifestation of renal tubular injury in lead intoxication and a comparison with patterns of aminoaciduria seen in other disease. *J. Pediat.*, 60:1-17.

Choie D D and Richter G W (1972 a) Cell proliferation in rat kidney induced by lead acetate and effects of uninephrectomy on the proliferation. *Am, J. Pathol.*, 66:265-276.

Choie D D and Richter G W (1972 b) Lead poisoning. Rapid formation of intranuclear inclusions. *Science*, 177:1194-1195.

Clasen R A, Hartmann J F, Starr A J, Coogan P S, Pandolfi S, Laing I, Becker R and Hass G M (1974) Electron microscopic and chemical studies of the vascular changes and edema of lead encephalopathy. *Am. J. Pathol.*, 74:215-240.

Columbano A, Ledda G M, Sirigu P, Perra T and Dani P (1983) Liver cell proliferation induced by a single dose of lead nitrate. *Am. J. Pathol.*, 110:83-88.

Conrad M E and Barton J C (1978) Factors affecting the absorption and excretion of lead in the rat. *Gastroenterology*, 74:731-741.

DeLuca J, Hardy C A, Burright R G and Donorick P J (1982) The effects of dietary fat and lead ingestion on blood lead levels in mice. *J. Toxicol. Environ. Health*, 10:441-447.

Duvenkamp A (1984) Karyokinetic reactions in rat kidney proximal convoluted segment epithelium on long-term lead supply with drinking water. *Acta. Anat.*, 119:121-123.

Furia T E (1968) Handbook of food additives. Chem. Rubber Publ. Co., Cleveland, Ohio, p. 263 and 289.

Garminati G M (1959) Protective action of liver extracts and vitamin B_{12} against the development of anemia produced by lead in rabbits. *Chem. Abstracts.* 53:15359b.

Goyer R A, Leonard D L, Moore J F, Rhyne B and Krigman M R (1970 a) Lead dosage and the role of the intranuclear inclusion body. *Arch. Environ. Health.*, 20:705-711.

Goyer R A, May P, Cates M and Krigman M R (1970 b) Lead and protein content of isolated intranuclear inclusion bodies from kidneys of lead-poisoned rats. *Lab. Invest.*, 23:245-251.

Hirsch G H (1973) Effect of chronic lead treatment on renal function. *Toxicol. Appl. Pharmacol.*, 25:84-93.

Hsu F S, Krook L, Shively J N and Duncan J R (1973) Lead inclusion bodies in osteoclasts. *Science*, 181:447-448.

Hunter B and Wobester G (1979) Encephalopathy and peripheral neuropathy in lead poisoned Mallard duck. *Avian Dis.*, 24:169-178.

Jones T C and Hunt R D (1983) Veterinary pathology. 5th ed., Lea & Febiger, Philadelphia, p. 1000.

Kennedy G Arnold D, Keplinger M L and Calandra J C (1971) Mutagenic and tetratogenic studies with lead acetate and tetraethyl lead. *Toxicol. Appl. Pharmacol.*, 19:370.

Keller C A and Doherty R A (1980) Effect of dose on lead retention and distribution in suckling and adult female mice. *Toxicol. Appl. Pharmacol.*, 52:285-293.

Klauder D S, and Petering H G (1977) Anemia of lead intoxication. A role for copper. *J. Nutr.*, 107:1779-1785.

Kostial K, Sigmonovic I and Pisonic M (1971) Lead absorption form the intestine in new born rats. *Nature, 233:564.*

Lefauconnier J M, Hauw J J and Bernard G (1983) Regressive or lethal lead encephalopathy in the suckling rat. *J. Neuropathol. Exp. Neurol.*, 42:177-190.

McLachlin J R, Goyer R A and Cherian M G (1980) Formation of lead induced inclusion bodies in primary rat kidney epithelial cell cultures : Effect of Actinomycin D and Cycloheximide. *Toxicol. Appl. Pharmacol.*, 56:418-431.

Michaslson I A (1973) Effects of inorganic lead on RNA, DNA and protein content in the developing neonatal rat brain. *Toxicol. Appl. Pharmacol.*, 26:539-548.

Miller G D, Massaro T F, Granlund R W and Massaro E J (1983) Tissue distribution of lead in the neonatal rat exposed to multiple doses of lead acetate. *J. Toxicol. Environ. Health. 11:121-128.*

Murakami M, Kawamura R, Nishi S and Katsunuma H (1983) Early appearance and localization of intranuclear inclusions in the segments of proximal tubules of rat following ingestion of lead. *Brit. J. Exp. Pathol.*, 64:144-155.

Mylroie A A, Moore L and Erogbogbo U (1977) Influence of dietary factors on blood and tissue lead concentrations and lead toxicity. *Toxicol. Appl. Pharmacol.*, 41:361-367.

Mykkanen H M, Dicerson J W T and Lancaster M (1980) Strain differences in lead intoxication in rats. *Toxicol. Appl. Pharmacol.*, 52:4141-421.

Pillmer L (1940) Vitamin C in chronic lead poisoning. *Am. J. Med. Sci.*, 200:322-327.

Pokotilenko G M (1964) Vitamin B_6 therapy in experimental poisoning with lead and benzene. *Chem. Abstracts*, 60:15038h.

Powell H C, Meyers R R and Lamert P W (1982) Changes in Schwann cells and vessels in lead neuropathy. *Am. J. Pathol.*, 109:193-205.

Richer G W, Kress Y and Cornwall C C (1968) Another look at lead inclusion bodies. *Am. J. Pathol.*, 53:189-215.

Richards M B (1945) Inbalance of Viotamin B factors. Pyridoxine deficiency caused by addition of aneurin and chalk. *Brit. Med. J.*, 1:433-439.

Rikans L E (1984) Influence of aging on the susceptibility of rats to hepatotoxic injury. *Toxicol. Appl. Pharmacol.*, 73:243-249.

Rosen J F (1983) The metabolism of lead in isolated bone cell populations : Interactions between lead and calcium. *Toxicol. Appl. Pharmacol.*, 71:101-112.

Silvestroni A and Balletta A (1965) The biosynthesis of nicotinamide nucleotides in the erythrocytes after lead intoxication. *Chem. Abstracts.* 63:4855h.

Six M M and Goyer M A (1972) The influence of iron deficiency on tissue content and toxicity of ingested lead in the rat. *J. Lab. Clin. Med.*, 79:128-136.

Smith C M, DeLuca H F, Tanaka Y and Mahaffey K R (1978) Stimulation of lead absorption by vitamin D administration. *J. Nutr.*, 108:843-847.

Stofen D (1974) Environmental lead and the heart. *J. Mol. Cell Cardiol.*, 6:285.

Stow H D, Goyer R A and Krigman M R (1973) Experimental oral lead toxicity in young dog. *Arch. Pathol.*, 95:106-116.

Vander A J, Mouw D R, Cox J and Johnson B (1979) Lead transport by renal slices and its inhibition by tin. *Am. J. Physiol.*, 236:F373-378.

Walton J R (1974) Intranuclear inclusions in the lead poisoned cultured kidney cell. *J. Pathol.*, 112:213-215.

Ziegler E E, Edwards B B, Jensen R I, Mahaffey K R and Foam S (1978) Absorption and retention of lead by infants. *Pediat. Res.*, 12:29-34.

LEGENDS FOR FIGURES

Fig. 2 Blood film from rat exposed to lead for 1 week. Leptocytes are present. New methyleneblue wright stain. X 1000.

Fig. 3 Blood film from rat exposed to lead for 3 weeks. This shows many reticulocytes. New methyleneblue wright stain. X 1000.

Fig. 4 Blood film from rat exposed to lead for 6 weeks. The red cells exhibit well marked anisocytosis, stomatocytosis, poikilocytosis. But reticulocytes are rare. New methyleneblue wright stain. X 1000.

Fig. 5 Proximal tubules of kidney from rat exposed to lead for 4 weeks. There are few cell debris in lumens of proximal tubules. Nuclei are hypertrophied. Hematoxylin and eosin (HE). X 200.

Fig. 6 Proximal tubules of kidney from rat exposed to lead for 4 weeks. Note some hypertrophied nuclei. Cytoplasms are irregularly stained by degenerating changes. H.E. stain. X 400.

Fig. 7 Medullary tubules of kidney from rat exposed to lead for 4 weeks. Nephrosis and nuclear pyknosis are seen. H.E. stain. X 400.

Fig. 8 Proximal tubules of kidney from rat exposed to thiamin 10mg and lead for 4 weeks. Proteinaceous hyaline casts within the tubular lumens are seen. Histologiical changes of the group are milder than lead group. H.E. stain. X 200.

Fig. 9 Medullary tubules of kindey from rat exposed to lead for 6 weeks. Severe interstitial fibrosis is seen. H.E. stain. X 100.

Fig. 10 Proximal tubules of kidney from rat exposed to lead for 6 weeks. Inclusion body is bordered by chromatin margination. H.E. stain. X 1000.

Fig. 11 Light microscopy of Epon-embedded section from kidney of rat exposed to lead for 6 weeks. Many intranuclear inclusion bodies and lysosomes are seen. Toluidine blue stain. X 400.

Fig. 12 Light microscopy of Epon-embedded section from kidney of rat exposed to lead for 6 weeks. The size and number of inclusion bodies are various. Toluidine blue stain. X 1000.

Fig. 13 Light microscopy of Epon-embedded section from kidney of rat exposed to thiamin 10mg and lead for 6 weeks. The size and number of inclusion bodies are decreased in comparision with lead group. Toluidine blue stain. X 200.

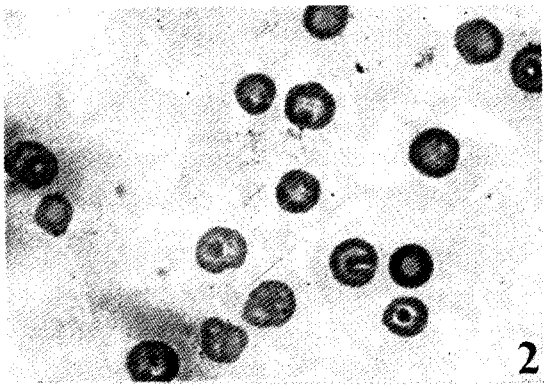
Fig. 14 Electron microscopy of renal proximal tubule cell from rat exposed to lead for 6 weeks. Two inclusion bodies are located on the low side of the nuc-

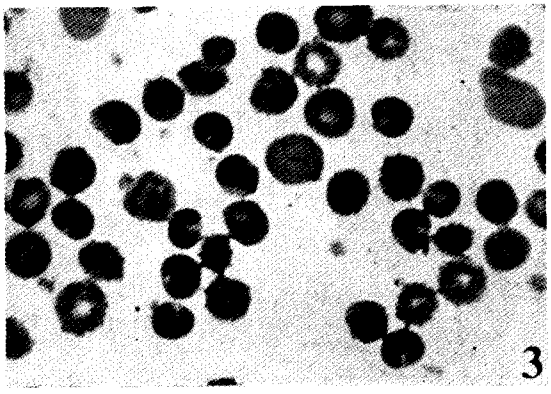
leolus (arrow). Section stained with uranyl acetate and lead citrate. X 5300.

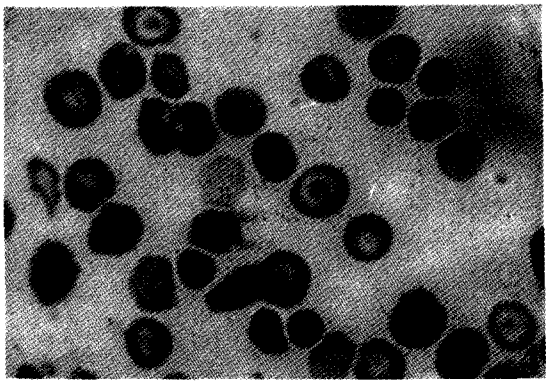
Fig. 15 Detail of inclusion body. The inclusion has a dense central core and outer fibrillar zone. X 12000.

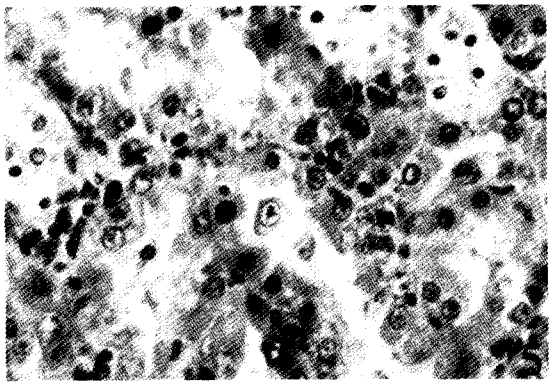
Fig. 16 Liver of rat exposed to lead for 3 weeks. Binucleated cells are seen and hepatocytes are ballooned. H.E. stain. X 400.

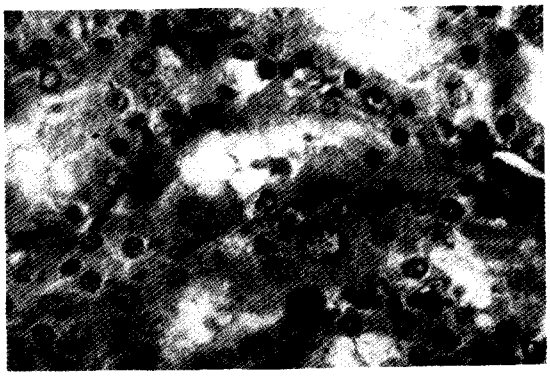
Fig. 17 Liver of rat exposed to lead for 4 weeks. Many vacoules are seen. Intranuclear inclusion bodies are rare. H.E. stain. X 200.

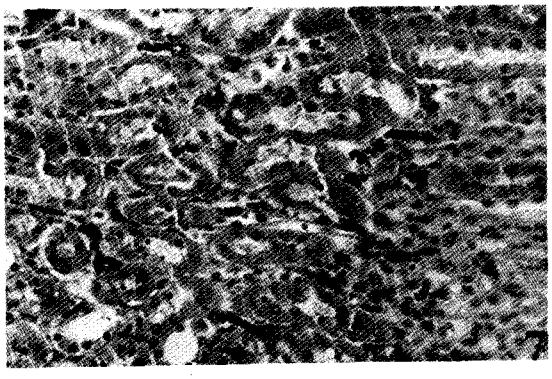

2

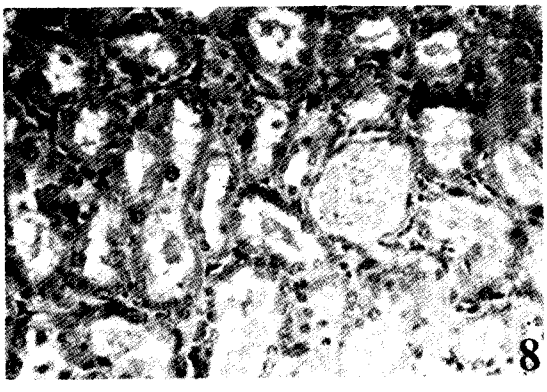

3










8