韓國臨床獸醫學會誌 第10卷 第1號
Korean J. Vet. Clin. Med.
Vol. 10, No. 1, June, 1993

Experimental Studies on Lead Toxicity in Domestic Cats

1. Symptomatology and Diagnostic Laboratory Parameters

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Abstract

Lead toxicity was evaluated in forty-five cats on a balanced diet, treated with 0(control), 10, 100(low), 1,000, 2,000, and 4,000(high) ppm of lead acetate orally on a body weight basis. The objectives were to establish toxic dosage level of lead in cats, to characterize changes in behavior and clinical pathology, and to demonstrate what blood lead concentrations correlate with the known dosages of lead.

Some high dose cats showed projectile vomiting, hyperactivity, and seizures. The growth rates did not appear to be altered in any of the dosed groups. Normal blood lead concentration in cats were lower than that of humans, dogs, and cattle. Blood lead concentrations of 3 to 20μg/100mℓ could be termed a "subclinical" range in the cat. Clinical lead toxicity in cats may have blood lead concentrations ranging 20 to 120μg/100mℓ. Zinc protoporphyrin concentrations were proportional to lead dosages and a significant ZPP elevation, greater than 50μg/100mℓ, may be indicative of clinical lead toxicity. The enzyme aminolevulinic acid dehydratase showed an inverss dose response relationship for all lead dosages and a significant ZPP elevation, greater than 50μg/100mℓ, may be indicative of clinical lead toxicity. The enzyme aminolevulinic acid dehydratase showed an inverse dose response relationship for all lead dosages and appears to be a good indicator of lead exposure in cats. Urinary aminolevulinic acid concentrations generally increased with lead dosage, but individual values varied. Hair lead concentrations rose proportionately to lead dosages.

Lead at least in high doses appears to inhibit chemotactic activity of polymorphonuclear cells and monocytes. No consistent dose response relationships were observed in hemoglobin, RBC, WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts. There were no consistent dose related changes in total protein, plasma protein, BUN, and ALT values. Reticulocyte counts did not increase significantly in most lead dosage levels, and are probably of little value in diagnosing lead toxicity in cats.

The fact that no significant changes were found in nerve conduction velocities may support that there was no segmental demyelination resulting from lead ingestion.

The lethal dose in cats appear to range from 60 to 150gm/Kg body weight.

A reliable diagnosis of lead poisoning can be made utilizing blood lead, ZPP, and ALAD, and hair lead.

Introduction

Animals and human beings are exposed to lead from many sources in the environment. Frequently reported sources are lead-based paint, plaster, dust

and dirt, ambient air resulting from burning of leaded gasoline, lead ore smelters, pottery, distilled liquor using lead tubing, linoleum, grease, roofing material, batteries, plumbing materials, putty, solder, golf balls, water from leaded pipes, and some glazed ceramics. Reported cases of clinical lead poisoning in cats have generally been associated with environmental exposure to dust, paint, and other materials. Scott[29], reported clinical lead poisoning in three cats in which elevated lead concentrations in soil were incriminated as a source from which the animals were obtaining the lead. A case of clinical lead poisoning in a cat was reported by Watson[36], in which the cat had lived for several years in an old house which the owners had recently begun to remodel by sanding paint from the walls. Watson presumed the cat ingested the old paint flakes and dust. In cats, lead-containing materials capable of contaminating the fur or paws might be very important because of their fastidious grooming behavior. Cats live in the same environment as man and are exposed to similar hazards.

To the author's knowledge there are no more clinical reports on the hazard to cat of ingestion of lead in foods. Only one study of experimental lead toxicity has been reported in cats, and it did not fully describe the dosages, clinical signs, diagnostic laboratory data for clinical estimations.

The purpose of this study was to evaluate the toxicity of feline lead ingestion at several dosage levels. The major objectives of this research were to establish both toxic and tolerated dosages of lead for cats based on clincical and laboratory parameters and to determine if subtle subclinical effects of lead infestion occur in cats, particularly effects on cellular immunity.

Materials and Methods

Experimental animals: The domestic cats used in this study were either purchased from Liberty Hill Laboratories(Liberty Corner, USA) (R-series) or obtained random source(L-series) from licenced animal breeders. Random source cats were conditioned for at least two weeks before being started on the project. All cats were vaccinated against feline panleukopenia(Felocel, Norden Lab., USA), feline rhinotracheitis and feline calici virus(Norden Lab., USA and FVR-C, pitman-Moore, Inc., USA). All cats were dewormed with dichlorvos(Task tabs, SQUIB by Diamond Shamrock Corp., USA).

Four cats in the same dosage group were housed in each run, which was constructed with a concrete floor and wire mesh walls with metal resting boards.

Cats were maintained in a room kept at 70 to 75 degrees F and approximately 40 to 45% relative humidity. The cats were provided with commercial feed(Purina Cat Chow, Ralston Purina Co., USA) and water ad libitum. Feed and water were periodically monitored for lead and levels maintained at $<.04$ ppm. Polycarbonate litter pans filled with clay absorbent litter were provided.

All blood samples for analytical procedures were taken by jugular venipuncture in sterile 6 or 12mℓ plastic syringes attached to a 20 gauge, 1 1/2 inch needle. Before bleeding, cats were anesthetized by intramuscular injection of 10:1:1 mixture of ketamine hydrochloride(Ketaset, 100mg/mℓ, Bristol Lab., USA), acepromazine maleate(10mg/mℓ), and atropine sulfate (1/120 grain/mℓ) at a dose of 0.1mℓ/lb body weight.

Lead administration: Cats were started on the project at 4 months of age and were dosed for 17 to 267 days. Forty-five cats were randomly divided into 3 groups:control, low dose, and high dose. Thirteen conditioned cats were used as a control group receiving food containing less than 4ppm lead. The low dose group was composed of 16 cats, of which 8 cats were treated with 10ppm and the other 8 cats were treated with 100ppm. The high dose group was composed of 16 cats, of which 8 cats were fed 1,000ppm, 4 cats were dosed 2,000 ppm, and 4 cats were treated with 4,000ppm of lead acetate(equal numbers of males and females) (Table 1). Treated groups received daily doses of lead acetate ground with a mortar and pestle, contained in gelatin capsules, orally.

The dosage was figured by the following formula: Dose = Average weight(kg) × 0.05(μg/kg) × 1.83 where 0.05(5%) represents the amount of feed consumed on a body weight basis per day and "1.83" was used to give the proper amount of lead that is in lead acetate(M.W. 379; Pb=207M.W.) because lead is 54.6%(1/0.54=1.83) of the formula weight.

Blood lead: Blood lead was determined before exposure and 1, 3, and 5 months after exposure. For blood lead analysis, blood was collected with heparin in lead free 15mℓ screw cap tubes. Quantitative analysis was performed by the methyl isobutyl ketone(MIBK) method. Lead was freed from the blood matrix by lysing the cells, complexed by ammonium pyrrolidine dithiocarbamate(APDC) and partitioned into methyl isobutyl ketone(MIBK).

Analysis was then done by atomic absorption spectrophotometry: In each of 6 screw cap tubes, 0, 1, 2, 3, 4, and 5mℓ of the working standards were placed, respectively(working standard: 2μg lead/mℓ; 2mℓ of the intermediate lead standard diluted to 100mℓ). Five mℓ of blood were placed in screw cap tubes and 1mℓ of 5% Triton x-100(TX) solution was added and the tubes mixed on a Vortex mixer until well hemolyzed. One mℓ of the APCD solution(2%) was added to each tube, followed by vortexing for 30~45 seconds. Ten mℓ of MIBK was added to the blank and standards. After extraction of lead into the MIBK, the standards represented 0, 0.2, 0.4, 0.6, 0.8, and 1.0μg lead/mℓ, respectively. Five mℓ of MIBK or 1mℓ of MIBK/mℓ of blood was added to the blood. Tubes were capped and shaken vigrously for 2 minutes and centrifuged for 5~10minutes at 2,000rpm. The organic supernatnat was aspirated form the screw cap tubes into a test tube, and the lead determined by an atomic absorption spectrometer set at 2833 nm wavelength, slit 4. A concentration curve was constructed from the standards absorption levels. The lead concentrations of the samples were read directly from this curve.

Zinc protoporphyrin (ZPP): Zinc protoporphyrin(ZPP) was determined before exposure, and 1, 3, and 5 months after exposure. For ZPP analysis, a drop of fresh heparinized blood was placed on a 25mm square glass coverslip. The blood was stirred on the cover slip at least 5 seconds and the slide was placed in the square recess of the slide carrier of a

Table 1. Lead Administration(ppm) for Each Experimental Group of Cats

Group	Daily dose	Cat ID	Sex	Months	Total dose
Control	0	L-14	M	8	0
	0	L-19	F	8	0
	0	L-20	M	8	0
	0	L-21	M	4	0
	0	L-22	M	5	0
	0	L-23	F	5	0
	0	L-24	M	5	0
	0	L-25	F	5	0
	0	L-26	F	5	0
	0	L-30	F	8	0
	0	RK-1	M	8	0
	0	RK-2	F	8	0
	0	RK-3	M	5	0
Low	10	L-12	F	8	2,450
	10	RI-4	F	8	2,450
	10	L-18	F	8	2,450
	10	L-11	F	8	2,450
	10	RH-4	M	9	2,670
	10	RE-4	M	9	2,670
	10	RB-2	M	9	2,670
	10	RJ-2	M	9	2,670
	100	RH-3	F	8	22,800
	100	RJ-3	F	9	25,100
	100	RL-1	F	8	23,500
	100	RN-2	F	9	26,700
	100	RO-4	M	8	23,000
	100	RG-4	M	7	21,400
	100	L-17	M	7	20,700
	100	L-15	M	7	20,500
High	1,000	RE-1	F	5	147,000
	1,000	RA-1	F	5	148,000
	1,000	L-13	F	1.5	48,000
	1,000	RD-3	F	5	147,000
	1,000	RO-5	F	2	66,000
	1,000	RM-4	M	5	158,000
	1,000	RH-6	M	5	158,000
	1,000	RG-5	M	5	141,000
	2,000	RK-4	F	3.5	206,000
	2,000	RA-3	F	4.5	270,000
	2,000	RO-3	M	3.5	220,000
	2,000	RJ-1	M	2	132,000
	4,000	RA-2	F	1	140,000
	4,000	RE-3	F	0.5	68,000
	4,000	RB-3	M	1	136,000
	4,000	RI-1	M	3	392,000

hematofluorometer(Hemafluor ZP, Haakebuchler Instruments Inc., USA). Using a glass pipette tip, the blood was spread to fully cover the area that would be in line with the fluorometer window opening. The slide carrier was gently pushed in until stopped by the first detent(background) position. After the first number went off(an indicator of proper instrument function), the slide carrier was further inserted to the second detent where another number was displayed to check if the exciter lamp was functioning. Finally the slide was inserted to the third stop(sample position) to display the concentration of ZPP in μg/100mℓ blood.

Aminolevulinic acid dehydratase (ALAD): Aminolevulinic acid dehydratase (ALAD) was determined before exposure and 1, 3, and 5 months after exposure. The ALAD assay was performed by the procedure of Tomokuni.[34)]

Heparinized blood was hemolyzed and combined with appropriate ALA reagents, incubated and then reacted with Ehrlich's reagent to allow color development. Activity was expressed as micromoles of porphobilinogen per hour per milliliter of red blood cells. Blood(3mℓ) was drawn into a heparinized syringe and immediately placed in an ice bath. Tests were run at pH level of 6.8. For each sample, 0.5mℓ of 0.2M phosphate buffer(pH 6.8) was added. Aminolevulinic acid(0.5mℓ of 0.02M) was added to all tubes, and the mixture was incubated in a water bath at 37℃ for 5 minutes. For duplicate runs, 1.0mℓ whole blood was hemolyzed by adding 6.50mℓ of distilled water and vortexing in an ice bath. The remaining whole blood was used for hematocrit determinations. Hemolysate(1.5mℓ) was added to each test tube in an ice bath. After hemolysate addition, the tubes were incubated at 37℃ for one hour in a water bath. After the one hour incubation, 1.0mℓ of TCA was added to all tubes to stop the reaction. The tubes were vortexed and centrifuged at 2,000×g at 4℃ for 10 minutes(Damon/IEC centrifuge, USA). The supernatant(1.0mℓ) from each tube was combined with 3.0mℓ of Ehrlich's reagent in an ice bath. The tubes were then removed from the ice bath and the color was allowed to develop for 10 minutes. Absorbance was read at 555nm(Beckman DB spectrophotometer, USA). Blanks were prepared by combining 1.0mℓ of distilled, deionized water with 3.0mℓ of Ehrlich's reagent (10 minute color development).

Activity was calculated using the following formula:

$$\text{Activity}=\frac{\%\ \text{Absorbance}}{\text{Hct}(\%)}\times\frac{70}{6.4\times10^{4}\ \text{l/mol cm}}\times\frac{10^{6}\ \mu\text{mol}}{10^{3}\text{m}\ell}$$

where activity is expressed as micromols of porphobilinogen(PBG) per hour per milliliter of red blood cells(the molar absorbancy of PBG was given as 6.4×10^{4} liter/mole cm) and 70 is the dilution factor.[34)]

Urinary d-aminolevulinic acid(ALA): Urinary ALA was determined by ion exchange column chromatography(Davis urinary ALA test; Bio-Rad Lab., USA). Urine samples were collected once or twice from 28 cats restrained with a ketamine-acepromazine-atropine mixture by adbominal paracentesis. The urine sample was placed in a sealed test tube, covered with aluminum foil and kept at −20℃ until determination. This test kit was provided with two sets of columns. The procedures are as follows: One blue tinted column(preparatory column) and one clear column(separatory column) were used for each sample. Columns were shaken vigorously to resuspend the resin. The resin was allowed to settle. First the cap of the clear column was removed and then the tip was snapped off, and placed in the rack and allowed to drain. Second, the cap of the blue tinted column was removed and then the tip was snapped off, placing the blue tinted column piggy-back fashion into the clear column. Approximately 10mℓ of distilled water was added to the top of each cloumn unit and allowed to drain through both columns. Urine(0.5mℓ) was pipetted into the top of the column unit and washed with approximately 10mℓ of distilled water. The wash was repeated twice(total 30mℓ). The preparatory column was discarded and the separatory column was re-

moved from the rack and placed in a numbered test tube and eluted with 7.0mℓ of 1.0M sodium acetate. The separatory column was discarded. Acetylacetone (0.2mℓ) was added to each of the test tubes and mixed well. The test tubes were placed in a boiling water bath(90℃ or greater) for 10 minutes, after which the samples were removed from the water bath and allowed to cool to room temperature. Ehrlich's reagent(7.0mℓ) was added to each test tube. The test tubes were allowed to stand for 15 minutes for complete color development. The optical density of the final reaction mixture was read at 553 nm in a standard laboratiory colorimeter(DB Spectrophotometer, Beckman Instrument Inc, USA). The concentration of urinary ALA in the unknowns was determined from the standard curve and expressed as mg/100mℓ.

Hair lead:Hair for lead analysis was taken from a defined area of the neck(brisket) measuring approximately 6 cm×12cm. Hair was clipped with a clean No. 40 electroic clipper blade and collected in clean plastic bags(Whirlpak, Nasco, USA). All hairs were analyzed by atomic absorption spectrometry at the trace substance Laboratory of University of Missouri.

Chemotaxis assays:Chemotaxis assays were carried out at 3 months after exposure. Chemtaxis assays were performed by the under-agarose method as follows:Ten to 12mℓ of blood were taken from the jugular vein of a cat into a syringe containing 0.2mℓ heparin plus 1.0mℓ of 6% Dextran T-500 in Lactated Ringer's. The blood was allowed to stand with needle end up at a 45 degree angle for one hour at room temperature. The female end of a butterfly needle with 6 inches of capillary hosing was attached and the leukocyte-rich plasma was expelled into a sterile 50mℓ(Corning) tube. The tube was placed in a 0℃ ice water bath. Cold RPMI-1640 medium was added to the plasma to a total volume of 20mℓ. The tube was centrifuged at 4℃ in the IEC refrigerated centrifuge at 300×g for 10 minutes. Supernatant was discarded and cells were resuspended in cold, fresh RPMI-1640 to a total volume of 5.0mls. Cold lymphocyte separation medium(LSM)(5.0mℓ) was placed in a sterile 50mℓ centrifuge tube. The tube was placed in an ice bath. Five mℓ cell suspension were carefully layered over the LSM floor with a sterile siliconized Pasteur glass pipette. The tube was centrifuged at 4℃, 250×g(IEC centrifuge) for 20 minutes. Monocytes on the interphase were suctioned off into a sterile siliconized pasteur pipette and transfer to another sterile 50mℓ centrifuge tube labeled "Monos". Cold RPMI-1640 was added into both tubes up to 20mℓ and both tubes were centrifuged at 4 degrees C(300×g) for 10 minutes. Supernatant was discarded from both tubes and cells(monocytes and PMN's) were resuspended in cold RPMI-1640 to a volume of 5.0mℓ. Fifteen mℓ of cold, sterile distilled water were added to PMN cells and the cells were agitated gently to lyse RBC's. Exactly 55 seconds later, 5.0mℓ of 3.6% sterile NaCl were added to the PMN tube to return suspension to an isotonic condition. The cells were counted on a hematocytometer(PMN's sometimes needed a 10:1 dilution in PBS). Ten μℓ of a PMN or monocyte cell suspension(1×10^8/mℓ RPMI) were placed in the middle wells punched into prepared agar(1% agarose, 0.5% gelatin in MEM) in 60mm×15mm tissue culture dishes(Falcon, B-D, Becton, Dickinson & Co. USA). Ten μℓ of chemoattractant-(zymosan activated serum[ZAS] or formylmethionyl-phenylalanine[FMP]) was placed in the outermost wells and RPMI media was placed in the innermost wells. The plates were incubated in an incubator for 4 hours at 37℃ with 5% CO_2. The plates were removed from the incubator and fixed with 5.0mℓ of 2.5% glutaraldehyde in PBS for 45 minutes at room temperature or at 4℃ overnight. The plates were drained and the agar was carefully flipped out. Filtered CAMCO quick stain(5.0mℓ) (Scientific products) was pipetted onto the plates. The plates were rotated(Fisher Clinical Rotator, USA) at 60 cycles/minute for 5 minutes, rinsed in a gentle stream of tap water, then finally rinsed in distilled deionized water and dried in an inverted position at a 45 degree angle. Migration distances from the cell well perimeter was measured

using a microprojector(Tri-Simplex, Bauch & Lomb, USA) image onto millimeter grid paper.

Directed migration was determined by the distance from the cell well perimeter to leading edge of the migrated cells toward chemoattractant well. The distance from cell well perimeter to leading edge of migrated cells towards the media well was considered random migration. The chemotactic difference was determined by subtracting the random migration from the directed migration. The chemotactic index was calculated by dividing the directed distance by the random distance. Each plate contained four cell wells: each chemoattractant was run in quadruplicate. The mean and standard deviation were calculated from the 4 replicates.

Complete blood count: CBC were performed before exposure and at 3 and 5 months after exposure. Complete blood counts were determined using automated procedures for cell counts(Coulter Counter, USA) and by direct visual examination of Wright's stained blood smears for differential leukocyte counts.

Reticulocyte counts: Reticulocyte counts were done before exposure and at 1, 3, and 5 months after exposure. Freshly drawn venous blood was aspirated up to the 0.5 line in a leukocyte diluting pipet. An equal volume of new methylene blue(new methylene blue powder 0.5gm+potassium oxalate 1.6gm+distilled water 100mℓ) was drawn up until the blood column reached the 1 mark. Blood and dye were moved back into the bulb, mixed well, and left stand for 15 minutes. A small drop of mixture was expelled on a slide and a standard blood smear was made. Using a 10mm square/100 squares micrometer grid eyepiece, reticulocytes were counted while examining 1,000 erythrocytes under oil immersion.

Clinical chemistry: Four clinical chemistry determinations(total protein, glucose, BUN, and SGPT/ALT) were made on each serum sample according to established techniques using a Kinetic Discreet Analyzer-(American Monitor, USA). Plasma protein was determined by refractometer(American Optical., USA). These determinations were done before exposure and at 3 and 5 months after exposure.

Nerve conduction velocity(NCV): NCV tests on the sciatic and ulnar nerves of cats were performed according to the method of Lee and Bowen[8] in thirty-two cats at the 6 month time point(2 high dose cats at 5 and 1/2 month point). These cats included 10 cats in the control group(4 cats were not otherwise on this project), 15 cats in the low dose group, and 7 cats in the high dose group. In all experiments, cats were anesthetized with pentothal IV, intubated anesthesia was maintained with halothane. Nerves were stimulated with single rectangular pulses of supramaximal strength and 0.1 millisecond in duration with a Grass Stimulater, Model S8C(Quincy, USA) and TECA monopolar teflon coated needle electrodes, Model MG 25(TECA Corp., USA). The sciatic nerve was stimulated proximally just caudal to coxofemoral joint and distally(tibial nerve) just above the tarsocrural(tibiotarsal) joint. The ulnar nerve was stimulated proximally at elbow and distally medial to accenssory carpal bone. Muscle evoked potentials were recorded from interosseus muscles using TECA monopolar needle electrodes. They were amplified by GRASS Preamp, Model p511 and displayed on Tektronix Storage Oscilloscope(Textronics, USA). Muscle evoked potentials were photographed with a polaroid camera after proximal and distal nerve sites were stimulated. The time difference in msec between onset of muscle evoked potentials from proximal stimulus and distal stimulus were measured from the photograph. The distance between proximal and distal stimulation sites on the animal was measured in mm. Nerve conduction velocity(NCV) was determined by the following formula:

$$\mathrm{NCV(meters/sec)} = \frac{\mathrm{distance(mm)}}{\mathrm{time(msec)}}$$

Statistical analysis: Analysis of variance(ANOVA) was run with all the parameters listed above via computer programs.[31]

The means and standard errors are presented in the Tables(see Results). The significant differences($p<.05$) are marked with asterisks.

Three month's values of 4 parameters were selected. Statistical analysis was performed to determine the relationship between the dependent variable, blood lead concentration(BPb) and a set of independent variables, zinc protoporphyrin(ZPP), aminolevulinic acid dehydratase(ALAD), and hair lead concentration(HPb). Our hypothesis was that there would be a positive relationship between BPb and ZPP and HPb and an inverse relationship between BPb and ALAD. Using these 4 parameters from 39 cats, regression analysis was carried out via computer programs.[31]

Results

Clinical observation: No demonstrable clinical signs were seen in control and low dose groups. Behavioral changes were observed in cats in the high dose group. They appeared to fear humans, often sitting at the far end of the run, and were reluctant to move. The high dose cats made more effort to escape from the grasp of the person trying to administer the lead capsules. Two 4,000ppm cats showed excessive salivation and projectile vomiting as a reflex when the person entered their run to give them the lead capsules. High dose cats were noticeably more nervous and hyperactive when handled for procedures such as bleeding or collection of hair samples. Several were observed having petit mal and/or grand mal seizures of 1 to 5 minutes' duration. Some of the high dose cats exhibited circling and other gait abnormalities. Ten cats were found dead, RA-2, RB-3 and RE-3(4,000ppm), RA-3, RK-4 and RJ-1(2,000ppm), L-13 and RG-5(1,000ppm), L-15(100ppm), and RI-2(10ppm).

Mean body weights are presented in Table 2. Pretrial mean weights of each dosage level were similar between groups, and all groups gained weight throughout the study. There did not appear to be an alteration of growth rates in any of lead dosed groups for the period studied.

Blood lead: Blood lead concentrations are presented in Table 3.

Mean and individual values for each group and each cat increased in proportion to the dosage of lead given. Cats fed 4,000ppm showed the most rapid increase, from 0.027 $\mu g/m\ell$ at pretrial to 1.925 $\mu g/m\ell$ at 1 month, while the 10ppm cats had blood concentrations that slowly rose from 0.028 $\mu g/m\ell$ at pretrial to 0.054 $\mu g/m\ell$ at 5 months. There were significant differences($p < .05$, ANOVA) in blood lead concentrations in high dose cats as compared to controls of the same month from the first measurement during the study(1 month) to the end of the study(> 5 months).

Zinc protoporphyrin(ZPP): Mean and individual zinc protoporphyrin values significantly increased($p < .05$, ANOVA), compared to the contols, in the 1,000ppm, 2,000ppm, and 4,000ppm cats in proportion to the

Table 2. Changes of Mean Body Weights(kg) of Each Experimental Groups

Group	ppm	Months							
		0	1	2	3	4	5	6	7
Control (n)	0	1.89 (13)	2.35 (13)	2.52 (13)	2.62 (13)	2.82 (13)	2.92 (13)	2.92 (12)	3.08 (11)
Low (n)	10	1.87 (7)	2.40 (8)	2.81 (8)	3.04 (8)	3.20 (8)	3.26 (8)	3.39 (8)	3.48 (8)
	100	1.95 (8)	2.56 (8)	3.03 (8)	3.24 (8)	3.35 (8)	3.44 (8)	3.93* (4)	3.47 (7)
High (n)	1,000	1.86 (8)	2.25 (8)	2.51 (8)	2.86 (7)	2.91 (7)	3.14 (7)	2.78 (5)	3.08 (5)
	2,000 & 4,000	1.86* (8)	1.81 (8)	2.30 (6)	2.68 (4)	2.75 (4)	3.20 (3)	3.50 (2)	4.40 (2)

*=Significantly different from controls of the same month at $p<0.05$ by ANOVA.

dosage of lead given(Table 3).

Aminolevulinic acid dehydratase (ALAD): Mean ALAD activity significantly decreased($p < .05$, ANOVA) in proportion to dosage in all dosed cats, even in 10ppm cats as compared to age matched controls. Mean ALAD activity is shown in Table 3.

The decline was sharpest in each group at 1month. The activity of control group increased until maturity, which was an expected finding in growing animals.

Urinary d-aminolevulinic acid(ALA): Urinary ALA was measured once or twice in 28 cats of different dosage groups. As shown in Table 4, urinary ALA concentrations showed no correlation with lead dosage levels. Urinary ALA concentrations in the control group have ranged from 0 to 3,110μg/100mℓ, ALA concentrations in the low dose group have ranged from 0 to 4,590μg/100mℓ, and ALA values in the high dose group have ranged from 0 to 7,130μg/100mℓ.

Hair lead: Mean hair lead concentrations increased in all dosage groups as compared to controls(Table 5).

In 10ppm cats, the highest mean hair lead value was 2.90μg/gm, at 3 months and decreased with time. In 100ppm cats, the highest mean hair lead value was 11.56μg/gm at 5 months of lead administration. The highest mean value in 1,000ppm cats was occurred at 5 months(91.50μg/gm), while in 2,000 and 4,000ppm cats, the highest mean value was noted at 3 months, then decreased slightly at 5 months.

Table 3. Changes of Blood Lead(μg/100mℓ)**, Zinc Protoporphyrin(μg/100mℓ Blood), and Aminovulinic Acid Dehydratase(μmol Porphobilinogen/hr/mℓ RBC) Obtained for Each Dose Level at Months 0, 1, 3, and 5 at 4 Different Doses in 5 Months

Group	ppm		Months (Mean±S.E.)			
			0	1	3	5
Control			(n=13)	(n=13)	(n=13)	(n=12)
		Blood Lead	1.0±0.7	ND	0.6±0.6	ND
	0	ZPP	18.08±1.52	16.77±1.52	17.92±1.39	16.92±1.56
		ALAD	.194±.011	.245±.018	.214±.019	.256±.008
Low			(n=8)	(n=8)	(n=8)	(n=8)
		Blood Lead	0.5±0.5	3.4±0.9	5.8±0.6	5.1±0.9
	10	ZPP	14.00±1.68	14.25±1.49	15.88±2.32	16.00±1.85
		ALAD	.211±.004	.146±.022*	.183±.022*	.168±.022*
			(n=8)	(n=8)	(n=8)	(n=8)
		Blood Lead	1.6±1.2	12.5±7.4	14.1±1.4	19.7±3.1
	100	ZPP	19.25±3.08	25.38±3.95	25.50±3.11	45.75±8.55
		ALAD	.187±.023	.080±.017*	.814±.013*	.081±.007*
High			(n=8)	(n=8)	(n=7)	(n=6)
	1,000	Blood Lead	1.4±1.4	68.6±19.4*	54.1±10.6*	76.7±17.1*
		ZPP	18.25±3.61	80.88±31.00*	314.14±104.67*	491.00±159.95*
		ALAD	.182±.025	.036±.005*	.032±.008*	.019±.003*,a
			(n=8)	(n=8)	(n=4)	(n=3)
	2,000	Blood Lead	0.6±0.4	125.9±31.7*	95.0±31.0*	91.7±41.3*
	4,000	ZPP	23.13±3.04	239.13±30.37*	741.00±225.77*	620.00±210.62*
		ALAD	.190±.021	.034±.008*	.030±.005*	.020±.001*

ND : None detected at the level of 0.03μg/mℓ : the value 0.01μg/100mℓ was assigned to enable statistical comparison.
* : Significantly different from controls of the same month at $p<0.05$ by ANOVA.
**:The unit "μg/mℓ" was converted to "μg/100mℓ" to compare with other studies.
a : n=5.

There were significant differences(p <.05, ANOVA) between controls and dosed cats at the pretrial measurement: at this point, all groups were significantly lower than controls. By the last hair lead measurement of the cats on this study(7 months), 1,000ppm and 2,000 & 4,000ppm cats showed a significant increase as compared to the control group. The control group hair lead value decreased in each time point during the trial measurements.

Chemotaxis assay:Chemotaxis assays of polymorphonuclear leukocytes and monocytes were carried out by the under-agarose method.

Both directed migration distance and random migration distance of the treated groups decreased in proportion with the increment of dose level of lead acetate(not shown). Correspondingly, the chemotatic indices in the treated groups increased in proportion to the lead dose(Table 6). Measurements of both PMN and monocyte chemotaxis were significantly different from controls(p <.05, ANOVA) in most of the high dose treatment.

CBC:hemoglobin values showed no significant differences(p <.05, ANOVA) among groups. However, the mean hemoglobin voulumes in 10ppm, 100ppm and 2,000ppm cats at month 3 were slightly higher than that of control while the mean hemoglobin in 1,000ppm cats was somewhat lower than that of the controls(Table 7).

There was a significant increase(p <.05, ANOVA) in mean red blood cell counts of 2,000 & 4,000ppm cats at 3 months compared to controls of the same month(Table 7).

Table 4. Changes of Urinary ALA(μg/100mℓ urine)* (Mean±S.E.)

Group	ppm	1st Trial	2nd Trial
Control	0	935±318 (n=10)	NA
Low	10 & 100	1207±362 (n=16)	1033±793 (n=4)
High	1,000 2,000 & 4,000	1586±858 (n=8)	1403±822 (n=4)

*=The unit "mg/100mℓ" was converted to "μg/100mℓ" to compare with other studies.
NA=Not applicable.

Table 5. Changes of Hair Lead level(μg/g) Obtained for Each Dose Level at Months 0, 3, 5 and 7 at 4 Different Doses in 5 Months

Group	ppm	Month (Meam±S.E.) 0	3	5	7
Control	0	1.90±0.26 (n=13)	1.75±0.11 (n=13)	0.61±0.07 (n=12)	0.51±0.10 (n=6)
Low	10	0.46±0.10* (n=8)	2.90±0.70 (n=8)	1.64±0.36* (n=8)	0.77±0.12 (n=8)
	100	0.65±0.13* (n=8)	6.11±0.68 (n=8)	11.56±1.73 (n=8)	7.40±1.01 (n=8)
High	1,000	0.45±0.09* (n=8)	48.88±8.81* (n=8)	92.50±9.85* (n=6)	15.50±1.44* (n=4)
	2,000 & 4,000	0.42±0.09* (n=8)	97.97±9.75* (n=7)	62.33±9.77* (n=3)	

*=Significantly different from controls of the same month at p<0.05 by ANOVA.

White blood cell counts showed a slight decrease(but not significantly different) in 10ppm, 100ppm, 1,000ppm and 2,000ppm cats in comparison with controls after 3 months(Table 7). There was a significant decrease of WBC(p <.05, ANOVA) in 10ppm cats and 1,000ppm cats at 5 months compared to controls of the same month.

Number of segmented neutrophils tended to decrease in both high and low dose groups throughout the experiment. A significant decrease(p <.05, ANOVA) compared to controls of the same month was found in neutrophil counts of 100ppm cats at 3 months and 10ppm and 1000ppm cats at 5 months(Table 8) and 1000ppm cats at 5 months(Table 8).

At pretrial, lymphocyte counts in the high dose group were significantly higher(p <.05, ANOVA) than controls. There was a significant decrease(p <.05, ANOVA) in lymphocyte counts in 10ppm cats at 5 months compared to controls of the same month(Table 8).

No significant differences(p <.05, ANOVA) were noticed with mean numbers of monocytes counted(Table 8).

Numbers of eosinophils in different dosage groups are demonstrated in Table 8. No significant differences(p <.05, ANOVA) were found between dosed groups and control group.

Means of MCV in 10ppm, 100ppm, 1,000ppm, 2,000ppm cats were lower than that of control group at all time points. There were significant differences (p <.05, ANOVA) between dosed groups and control group of the same month at pretrial and at 5 months(Table 7).

Means of MCH in the high and low dosage groups were lower than that of controls at all time points. There were significant differences (p <.05, ANOVA) in MCH of 100ppm, 1,000ppm, and 2,000 & 4,000ppm cats at pretrial and at 3 months. A significant decrease was also found in 1,000ppm cats at 5 months compared to controls of the same month(Table 7).

Mean values of MCHC in dosed groups were generally higher than that of the control group. There was a signifcant increase(p <.05, ANOVA) in MCHC of 10ppm cats at pretrial and at 3 months compared to controls of the same month(Table 7).

Reticulocyte counts: Means and standard errors of reticulocyte counts are demonstrated in Table 7. There were significant increases(p <.05, ANOVA) in reticulocyte counts of 1,000ppm cast at 5 months and 2,000 & 4,000ppm cats at 3 months compared to controls of the same month.

Clinical chemistry: Total protein tended to increase in control, 10ppm and 100ppm cats, but slightly de-

Table 6. Phagocyte Chemotaxis under Agarose Chemotaxis Indices

Group (ppm)	Mean±S.E. PMN : Serum	PMN : ZAS	Mono : Serum	Mono : ZAS
Control (0)	2.654±.081 (n=12)	3.178±.111 (n=12)	1.935±.025 (n=10)	1.964±.031 (n=10)
Low (10)	2.576±.070 (n=8)	3.005±.104 (n=8)	1.949±.020 (n=8)	2.063±.030 (n=8)
Low (100)	2.558±.096 (n=8)	3.343±.138 (n=8)	2.028±.041 (n=8)	2.270±.070 (n=7)
High (1,000)	3.142±.156 (n=5)	4.045±.033* (n=5)	2.375±.163* (n=5)	2.678±.141* (n=5)
High (2,000 4,000)	3.726±.096* (n=2)	4.186±.120 (n=2)	2.398±.150 (n=2)	3.074±.383* (n=2)

*=Significantly different from controls at p<0.05 by ANOVA.

PMN=polymorphonuclear cells(neutrophils); Mono=monocytes; ZAS=zymosan activated serum

creased in 1,000ppm and 2,000ppm cats. There was a significant decrease($p < .05$, ANOVA) in 2,000 & 4,000ppm cats at 3 months compared to controls at same month(Table 9).

Plasma protein values are presented in Table 9. There were no significant differences($p < .05$, ANOVA) in between dose groups and controls.

There was a significant difference($p < .05$, ANOVA) in glucose levels in 10ppm cats at 3 months as compared to controls of the same month(Table 9).

Table 7. Changes of RBC($10^6/mm^3$), Hemoglobin(gm/dℓ), Mean Corpuscular Volume(MCV; μ^3), Mean Corpuscular Hemoglobin(MCH; $\mu\mu g$), Mean Corpuscular Hemoglobin Concentration(MCHC;gm%), and Reticulocyte Counts(#/1,000 RBC Counted) Obtained for Each Dose Level at Months 0, 1, 3, and 5 at 4 Different Doses in 5 Months

Group	ppm		Month (Mean±S.E.)			
			0	1	3	5
Control	0		(n=13)	(n=13)	(n=13)	(n=12)
		RBC	6.15±0.37	ND	7.62±0.23	7.73±0.17
		Hemoglobin	8.9±0.47	ND	11.1±0.44	11.2±0.26
		MCV	46±1.2	ND	42±0.6	44±0.9
		MCH	14.6±0.2	ND	15±0.3	15.0±0.3
		MCHC	32.4±0.5	MD	35.0±0.3	34.6±0.4
		Reticulocyte	2.3±0.3	1.2±0.3	0.8±0.3	1.0±0.2
Low	10		(n=8)	(n=8)	(n=8)	(n=8)
		RBC	6.88±0.37	ND	8.32±0.26	8.01±0.16
		Hemoglobin	9.4±0.64	ND	11.8±0.53	11.3±0.42
		MCV	41±1.1*	ND	40±1.10	40±1.1*
		MCH	13.8±0.3	ND	13.2±0.4	14.5±0.4
		MCHC	34.3±0.5*	MD	38.1±1.6*	35.6±0.3
		Reticulocyte	0.9±0.2	1.6±0.5	1.5±0.5	0.9±0.1
	100		(n=8)	(n=8)	(n=8)	(n=8)
		RBC	6.00±0.35	ND	8.43±0.23	8.00±0.26
		Hemoglobin	8.1±0.39	ND	11.6±0.49	10.9±0.53
		MCV	42±1.1*	ND	41±1.1	40±1.4*
		MCH	13.7±0.4*	ND	13.8±0.5*	14.2±0.5
		MCHC	33.3±0.5	MD	34.8±0.4	34.9±0.5
		Reticulocyte	0.9±0.2	1.4±0.3	1.4±0.3	1.0±0.0
High	1,000		(n=8)	(n=8)	(n=7)	(n=6)
		RBC	5.94±0.36	ND	7.51±0.38	7.76±0.37
		Hemoglobin	8.2±0.49	ND	10.4±0.34	10.4±0.37
		MCV	42±0.7*	ND	41±1.1	39±1.2*
		MCH	13.7±0.2*	ND	13.9±0.5*	13.9±0.4*
		MCHC	33.5±0.5	MD	35.0±0.3	35.4±0.5
		Reticulocyte	0.9±0.4	1.6±0.3	1.0±0.0	2.3±0.6*
	2,000 & 4,000		(n=8)	(n=4)	(n=4)	(n=2)
		RBC	6.50±0.43	ND	9.26±0.78*	7.54±0.30
		Hemoglobin	8.5±0.36	ND	11.4±0.55	9.9±0.55
		MCV	40±0.9*	ND	40±1.3	39±1.0*
		MCH	13.2±0.3*	ND	13.1±0.4*	13.7±0.3
		MCHC	34.8±1.4*	MD	36.8±2.4	35.0±0.4
		Reticulocyte	1.1±0.2	1.8±0.4	2.5±1.0*	1.7±0.3

*=Significantly different from controls at $p<0.05$ by ANOVA.

ND : Not determined

Table 8. Changes in WBC(/mm³), Neutrophils(/mm³), Lymphocytes(/mm³), Monocytes(/mm³), and Eosinophils(/mm³) Obtained for Each Dose Level at Months 0, 3, and 5 at 4 Different doses in 5 Months

Group	ppm		Month (Mean±S.E.) 0	3	5
			(n=13)	(n=13)	(n=12)
Control	0	Total WBC	14,777±2.043	27,277±2,1388	26,800±2,030
		Neutrophils	7,934±1.092	16,458±2,051	17,376±1,848
		Lymphocytes	4,133±698	7,853±810	7,119±706
		Monocytes	640±192	816±199	359±83
		Eosinophils	923±226	1,557±313	1,536±299
			(n=8)	(n=8)	(n=8)
Low	10	Total WBC	14,525±2,368	23,900±1,648	16,587±2,392*
		Neutrophils	10,361±1,593	14,255±1,233	11,391±1,727*
		Lymphocytes	3,116±857	7,105±1,154	3,639±662*
		Monocytes	452±184	667±178	474±86
		Eosinophils	1098±588	1,462±317	1,007±188
			(n=8)	(n=8)	(n=8)
	100	Total WBC	19,550±1,896	23,100±1,556	21,900±1,222
		Neutrophils	9,996±1,438	10,910±787*	13,293±856
		Lymphocytes	6,903±1,398	9,908±993	5,495±618
		Monocytes	567±102	382±102	513±115
		Eosinophils	1,095±198	1,544±179	2,241±434
			(n=8)	(n=7)	(n=6)
High	1,000	Total WBC	20,637±3,870	25,043±2,696	18,300±3,319*
		Neutrophils	9,121±1,664	13,056±2,351	9,481±2,385*
		Lymphocytes	8,857±2,154*	9,252±1,465	7,006±1,538
		Monocytes	339±96	626±218	488±168
		Eosinophils	1,841±737	1,850±204	1,218±225
			(n=8)	(n=4)	(n=2)
	2,000 & 4,000	Total WBC	21,325±3,870	20,9502,777	23,250±1,650
		Neutrophils	10,311±1,568	11,4672,403	16,733±548
		Lymphocytes	9,443±2,148*	9,491±1,673	4,343±887
		Monocytes	607±288	645±359	498±498
		Eosinophils	902±233	1,400±444	1,677±813

*=Significantly different from controls at $p<0.05$ by ANOVA.

There was a significant decrease(p <.05, ANOVA) in blood urea nitrogen(BUN) levels in 10ppm cats at 5 months while there was a significant increase(p <.05, ANOVA) in 1,000ppm cats at 5 months(Table 9).

There was a significant difference(p <.05, ANOVA) in alanine aminotransferase(ALT) activity in 10ppm cats at 3 months as compared to controls at the same month(Table 9).

Nerve conduction velocity(NCV): There were no significant differences (p <.05, ANOVA) in nerve conduction velocity tests between the treated groups and the control group(Table 10).

Statistical analysis: Three month's values of 4 parameters were selected. Statistical analysis was performed to determine the relationship between the dependent variable, blood lead concentration(BPb) and a set of independent variables, zinc protoporphyrin(ZPP), aminolevulinic acid dehydratase(ALAD), and hair lead concentration(HPb). Our hypothesis was that there would be a positive relationship between

Table 9. Changes of Total Protein(gm/dℓ), Plasma Protein(gm/dℓ), Blood Glucose(mg/dℓ), Blood Urea Nitrogen(mg/dℓ), and ALT(μmol/min/liter) Obtained for Each Dose Level at Months 0, 3, and 5 at 4 Different Doses in 5 Months

Group	ppm		Month 0	Month 3	Month 5 (Mean±S.E.)
			(n=13)	(n=13)	(n=13)
Control	0	Total protein	5.8±0.3	6.5±0.2	6.8±0.2[a]
		Plasma protein	5.3±0.2	6.6±0.2	6.5±0.2
		Glucose	103±4.7	89±5.6	96±4.9[a]
		BUN	27±1.1	28±1.1	28±0.9
		ALT	47±9.4	38±3.9	41±2.9
	10	Total protein	6.1±0.3	6.9±0.1	6.8±0.2
		Plasma protein	5.9±0.3	7.1±0.1*	6.7±0.1
		Glucose	102±5.3	73±5.3*	94±3.9
		BUN	28±1.8	25±1.1	25±1.1*
		ALT	43±5.3	65±15.9*	44±5.3
	100	Total protein	5.8±0.2	6.6±0.1	7.0±0.2
		Plasma protein	5.7±0.2	6.7±0.1	6.7±0.1
		Glucose	97±5.3	86±3.5	98±3.9
		BUN	29±1.1	26±1.4	26±1.1
		ALT	32±3.9	37±1.8	40±3.9
			(n=8)	(n=7)	(n=6)
High	1,000	Total protein	5.6±0.2	6.5±0.2	6.7±0.2
		Plasma protein	5.7±0.2	6.5±0.2	6.6±0.2
		Glucose	111±6.7	82±3.0	90±4.9
		BUN	28±1.1	26±1.5	32±2.0*
		ALT	31±2.1	40±2.3	41±4.9
			(n=8)	(n=4)	(n=2)
	2,000 & 4,000	Total protein	6.1±0.2	5.7±0.2*	6.4±0.7
		Plasma protein	5.9±0.1	6.2±0.3	6.6±0.6
		Glucose	106±3.0	99±5.8	80±0.5
		BUN	28±0.6	27±1.2	26±1.5
		ALT	50±6.6	38±5.1	45±11.5

*=Significantly different from controls at $p<0.05$ by ANOVA.

[a] : n=2

Table 10. Nerve Conduction Velocity(NCV, meters/second) (Mean±S.E.)

Group	ppm	Sciatic Nerve	Ulnar Nerve
Control	0	111.18±3.66 (n=10)	91.20±2.68 (n=10)
Low	10	118.18±4.09 (n=8)	90.67±4.50 (n=8)
	100	116.27±6.14 (n=7)	93.87±2.81 (n=7)
High	1,000	122.96±7.75 (n=5)	84.18±4.24 (n=5)
	2,000 & 4,000	98.18±2.92 (n=2)	103.61±20.90 (n=2)

Table 11. The Hypothesized Relationships

Dependent variable	Independent variables	Hypothetic signs
	ZPP	+
BPb	ALAD	−
	HPb	+

BPb and ZPP and HPb and an inverse relationship between BPb and ALAD:

Using these 4 parameters from 39 cats, regression analysis(Neter and Wasserman, 1974) was done via computer programs(Statistical Analysis System, 1982). The estimated linear relationship was:

$$BPb\ i=0.1255+0.0009\ ZPP\ i-0.4401\ ALAD\ i+0.0021\ Hpb\ i$$
$$(0.00013) \quad (0.19887) \quad (0.00131)$$

$R^2=0.9233$, $F=140.39$, $i=1,2,3,\cdots 39$;"( )" indicates the standard error of the estimated coefficient.

The R square indicates that 92.33% of total variation in BPb was explained by the set of independent variables. Also, the validity of this model was supported by the high F statistic at a 1% significance level. The coefficients of ZPP and ALAD were different from zero at 5% significance level. The t-statistic of HPb indicated the coefficient was not significantly different from zero at the same significance level, but it was marginally different from zero at 10% significance level.

The signs of estimated coefficients were identical to the hypothesized signs for all three independent veriables. Based upon the regression results and appropriate statistical tests, a linear relationship was suitable to explain the variation in the dependent variable, BPb.

Discussion

Some of our high lead dose cats showed projectile vomiting, hyperactivity, and seizures. Our clinical observations were similar to those reported for children and dogs. Changes in behavior, especially seizures, associated with lead toxicity have been reported in children[6,9,13,15] and dogs.[10,24,39]

In naturally occuring lead toxicity in cats, Watson[36] observed anorexia and sporadic vomiting. Seizures have been observed in some cases of lead encephalopathy in cats.[19]

The growth rates(body weights) of our cats did not appear to be altered in any of lead dosed groups. Similarly, growth rates of dogs fed lead with a balanced diet,[24] did not show consistent changes as compared to the control dogs. In contrast, in an oral lead ingestion study of young dogs fed a calcium-and-phosphorus-low purified diet[32], growth of lead-fed dogs was significantly less than the control dogs.

Blood lead analysis is currently accepted as the best single laboratory test for diagnosis of increased lead absorption and lead poisoning. Blood lead is usually determined with whole blood by atomic absorption spectrophotometry.

The average blood lead concentration in the control group in this study, less than 1 μg/100mℓ, was lower than the average blood lead concentration of normal cats, 5.2 μg/100mℓ, reported by Bloom et al.[3] This difference may be due to the fact that the experimental animals used in our study were probably younger than in Bloom's study("all ages") and therefore had less exposure to environmental lead. The cat normal blood lead values generally appear lower than normal human blood lead values, 0 to 35 μg/100mℓ. Our cat normal values were lower than normal values of cattle, 6.3 μg/100mℓ, measured by Ruhr.[27]

From our study, blood lead concentrations can reach as high as 69±19 μg/100mℓ to 126±21 μg/100mℓ in cats, similar to people hospitalized with lead toxicity who have had blood lead values greater than 80 μg/100mℓ.[20]

Blood lead concentrations of approximately 3~20 μg/100mℓ could be termed a "subclinical" range in the cat, since none of the cats(10 and 100ppm) having

blood lead concentrations in that range showed any detectable clinical signs.

Cats with 10ppm dosage had blood lead concentrations in the same range as Bloom et al.[3] normal cat range. Clinically significant concentrations for cats would thus be between 20 and 120μg/100mℓ, since cats in the 1,000ppm(> 50μg/100mℓ) had multiple clinical and laboratory parameter abnormalities. For cats on a balanced(normal calcium and normal protein) diet, lead concentrations in the diet of less than 10ppm may be considered safe. However, in 2 dogs on a low calcium diet fed 5ppm lead, Hamir et al.[10] observed blood lead concentrations of 112 and 296μg/100mℓ, respectively(where 2 control dogs showed 28 and 33μg/100mℓ). The effect of a low calcium diet on lead absorption has not been studied in cats. The blood lead level is usually diagnostic of lead poisoning when the lead concentration is greater than 60μg/100mℓ in humans and dogs.[38] A blood lead level of 35μg/100mℓ was recommended as the upper safe level for children and dogs.[40] Dogs suffering from lead poisoning may have blood lead levels reaching 80～500μg/100mℓ.[29,37] The average blood lead levels in normal dogs and cats have been reported as 6.4μg/100mℓ and 5.2μg/100mℓ, respectively.[3]

However, Zook et al,[40] reported a mean blood lead level of 19±8μg/100mℓ for normal dogs and 94±64μg/100mℓ as the level for lead toxicity in dogs. In an experimental toxicity study in 8-month-old dogs that were fed a lead salt mixture(lead chloride:lead bromide:lead sulphate=1:2:1) at a dose rate of 50, 150, 300, and 600ppm to respective groups, blood lead concnetration in lead intoxicated dogs with high-fat-low-calcium diet was 100μg/100mℓ, whereas that with the commercial balanced diet was less than 50μg/100mℓ.[10] In young dogs(4 to 5 months old) with experimentally induced lead toxicity(1, 10, and 100ppm lead acetate for 10 months and 400ppm lead acetate for 3 months), there was a dose related response to blood lead concentration, and the clinically toxic level was found to be 60μg/100mℓ or more[24]. Staples[30] reported that dogs with a blood lead level of over 100μg/100mℓ would show clinical symptoms of lead poisoning. Allcroft[2] reported blood lead values ranging from 5 to 25μg/100mℓ(mean value; 13μg/100mℓ) as normal levels in goats, sheep, horses, cows, and calves. Ruhr[27] reported average normal blood lead value of 6.3μg/100mℓ(SD=2.8) in cattle in Missouri. In the same report, he suggested that 15μg/100mℓ is threshold level in cattle. In acute bovine lead poisoning, the blood lead concentrations ranged from 35 to 240μg/100mℓ(0.35 to 2.4mg/liter).[11,12]

Allcroft and Blaxter[1] induced lead toxity in ewes, which were dosed 100 to 200mg lead acetate per kg of dry matter ingested for 12 months. In their study, blood lead concentrations in ewes ranged from 22 to 34μg/100mℓ(0.22 to 0.34mg/liter). Cantarow and Trumper[5] considered, in people, blood lead values above 80μg/100mℓ should be regarded as intoxication. Normal values of blood lead concentrations of man are 0～35μg/100mℓ.

The determination of ZPP is also an assay in whole blood, utilizing the inherent fluorescence of ZPP molecule for measurement.

The average ZPP concentration in the control group in this study, 17.4μg/100mℓ, was similar to the average value, 19.6μg/100mℓ, in control dogs measured by Osweiler[24] and also similar to the average value, 21.56μg/100mℓ, in normal cattle.[27] In Ruhr's study, even though he measured free erythrocyte protoporphyrin(FEP), actually FEP might be considered as ZPP.[16] To the author's knowledge, no other published ZPP values in cats are available for comparison to our study. Our normal ZPP concentrations in cats were somewhat lower than that of healthy people, 20～59μg/100mℓ.[35] From our study, cats suffering from lead toxicity(> 1,000ppm) had ZPP values ranging from 80.88±31.00μg/100mℓ to 741.00±225.77μg/100mℓ, while ZPP values in occupational lead poisoning in humans ranged from 60 to 200μg/100mℓ.[35]

In our study, zinc protoporphyrin values were proportional to lead dosages and a significant ZPP eleva-

tion was indicative of a lead exposure of greater than 1000ppm(values were significantly increased compared to controls). However, ZPP concentrations in dogs did not change in a dose related manner.[24] The lack of change in ZPP concentrations in lead toxic dogs may be due to a difference in the configuration of the heme molecule between humans and dogs. This could cause an alteration in the optimal emission wave length for the assay in dogs. In cats, the assay appears to have good potential for lead diagnosis or screening.

Aminolevulinic acid dehydratase(ALAD), which catalyses the synthesis of porphobilinogen(PBG) from two molecules of ALA, is largely contained in erythrocytes. In humans, this enzyme is very sensitive to lead and its activity may be inhibited even before measurable deviations occur in other tests. In lead toxicity, ALAD activity decreases while the ALA level increases.[34]

In an experimental study in dogs, ALAD values decreased in relationship to increasing lead dosages. Mean ALAD activity in normal dogs was 19.6 μmol PBG/hr/mℓ RBC, whereas activity in lead intoxicated dogs fed a high dose of 1,000ppm orally was below 10 μmol PBG/hr/mℓ RBC. In normal cattle in Missouri, the average ALAD activity was 45.8 μmol PBG/hr/mℓ RBC [45.8unit(SD=20.6)].[27]

The average ALAD activity in the control group in this study, 0.228 μmol PBG/hr/mℓ RBC. was approximately 100 times lower than the average ALAD activity in control dogs, 30.85 μmol PBG/hr/mℓ RBC. as measured by Osweiler.[24]

Cat normal values appear only slightly lower than the activity of humans, 0.300~1,100 μmol /hr/mℓ RBC. at a BPb level of 10~20 μg/100mℓ, as measured by Tomokuni.[34] From our studies, cats suffering from clinical lead toxicity may have ALAD activities as low as 0.020 to 0.036 μmol PBG/hr/mℓ RBC(BPb 54.1~125.9 μg/100mℓ), whereas ALAD activity in humans with occupational lead poisoning(Bpb>0.40 μg/mℓ) have ranged 0.050 to 0.300 μmol/hr/mℓ RBC.[30,34] The enzyme ALAD showed an inverse dose response relationship for all lead dosages(even in 10ppm) and appears to be a good indicator of lead exposure in cats.

Aminolevulinic acid(ALA) is filtered by the kidney and excreted in urine.[33,36]

Increased ALA content in the urine has been shown to be an early manifestation of lead intoxication in humans(both adults and children).

In our study, urinary aminolevulinic acid(ALA) concentrations did not statistically correlate with lead dosage levels, although there was an average increase in ALA proportional to lead dose. Individual cat ALA concentrations varied widely. The average ALA concentration in the control group, 935 μg/100mℓ, was similar to the average value that Watson[36] measured in normal dogs and cats, 637 μg/100mℓ(38umol/ℓ). Average ALA values in low and high dose groups(1,207 and 1,586 μg/100mℓ, respectively) were much higher than average ALA value, 394.2 μg/100mℓ, that McSherry et al.[22] observed in "chronically sick" cats (not diagnosed as lead poisoning). Our ALA values in dosed groups were also generally higher than average ALA concentrations, 20~960 μg/100mℓ(normal 0~300 μg/100mℓ) of cattle with experimental lead toxicity.[22] In our study, ALA concentrations in dosed groups were higher than ALA concentrations 274.7 μg/100mℓ(normal 120~190 μg/100mℓ), in dogs with experimental lead toxicity.[22]

If future studies are planned to study lead toxicity in cats, it could be worthwhile to repeat ALA measurements since our varied results may be due at least in part to the use of expired dated columns of the kits.

Determination of lead in scalp hair may be a diagnostic aid in chronic or mild lead intoxication in children, particularly when other clinical or laboratory evidence is unavailable, or the period of lead exposure has already passed. Hair may accumulate and store lead for variable periods of time. As the hair continues to grow, the concentration of lead rises in relation to its distance from the scalp. Since human

scalp hair grows at a fairly constant rate of approximately 0.4mm/day(in children) it is possible to estimate the time and duration of exposure.[15]

Our dosed cat hair lead concentrations rose proportionately to lead dosages given. The average hair lead concentration in the control group in this study, 1.4 μg/gm was similar to the average hair lead concentrations of normal dogs, 1.7 μg/mg, as measured by Osweiler.[24]

Kopito et al.[15] found average hair lead values of healthy children, to be 31 μg/gm(2~92 μg/gm).

In human children and adult without known lead exposure, hair lead content ranged from 1 to 92 μg/gm of hair with a mean concentration of 20 to 31 μg/gm.[28]

Our cats in high dose group had hair lead concentrations as high as 41~100 μg/gm. This range appeared to be lower than one report of children, under 8 years of age with chronic or acute lead poisoning, of which the mean value was 276 μg/gm(ranging from 70~975 μg/gm).[15] Our dosed cat hair lead concentrations(41~100 μg/gm) was considerably higher than that of lead toxic dogs, 10.3 to 17.4 μg/gm, measured by Osweiler.[24] In his study, hair lead values in dogs changed proportionately to lead dosages. In contrast, Zook et al.[40] failed to find a dose related change in an experimental dog lead toxicity study. This contradiction between dog studies may be due to the fact that the canine hair does not grow continuously, and dog hair is shed seasonally. It is most likely that cat hair, which also does not grow continuously, would vary seasonally also.

Since both directed and random chemotaxis of neutrophils and monocytes generally decreased in proportion to the dose levels of lead acetate, lead at least in high doses appears to inhibit chemotactic activity of polymorphonuclear cells(PMN's) and monocytes.

They mentioned glucose may be lost into the urine due to lead induced damage of the proximal convoluted tubules where glucose should be reabsorbed. The reported no changes in BUN. Clinicopathological changes such as anemia, increased BUN level, increased RBC fragility, and intense normoblastic erythroid hyperplasia of the bone marrow were reported associated with lead toxicity in cattle.[7]

Although hemoglobin values tended to decrease with higher doses of lead, most values were not significantly different from controls. Thus in cats, hemoglobin synthesis does not appear significantly impaired even though increased ZPP concentrations would indicate that lead does affect the heme synthesis pathway. No dose response relationships were observed in RBC, WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts. There were no dose related changes in total protein, plasma protein, glucose, BUN, and GPT values. Osweiler[24] found no consistent changes attributable to lead in clinical blood parameters, such as hematocrit, hemoglobin, RBC, WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts. In lead toxicity study in dogs,[40] PCV was normal, hypochromia was noticed, numbers of circulating nucleated red cells varied directly with the amount of lead in the blood and hyperplasia of erythroid elements in the bone marrow.

Bond and Kubin[4] reported severe anemia in dogs which was mostly of the hypochromic macrocytic type. They postulated that lead causes hemolytic anemia due to the increase of fragility of red cells, resulting in a large number of normoblasts and reticulocytes appearing in the peripheral blood from the bone marrow as a compensatory mechanism.

Most reticulocyte counts in the dosed cats were not significantly different from the control group. The differences in our cats were relatively small(0.1~0.2%) and inconsistent compared to reports by other investigators of other species(>1.6% in dogs,[40] 1.1~5.9% in humans[23])

In our study, basophilic stippled RBC's were not found. In other species, some authors reported basophilic stippling in the RBC but others faild to find basophilic stippling. Bond and Kubin[4] found basophilic stippled red cells in dogs with clinical lead poisoning. Donawick[7] observed basophilic stippling

in a cow with chronic clinical lead toxicity. In contrast, Staples[30] reported basophilic stippling was not a consistent feature of lead toxicity in dogs. Jensen et al.[14] have demonstrated with electron microscopy that basophilic stippled red cells are reticulocytes. No reticulocyte counts in lead intoxicated cats were available to compare with our study. Since we found no basophilic stippling in our study, this parameter is probably of little value in diagnosing lead toxicity in cats.

From our nerve conduction velocity test, there were no statistically significant differences in NCV of the sciatic and ulnar nerves between the treated groups and the control group(although several cats in the higher dose group showed slightly decreased NCV velocity). Thus the author postulates that lead does not cause segmental demyelination in cats in the manner and dose given in our study. Fullerton[8] reported segmental demyelination and axonal degeneration of peripheral nerve of guinea pigs by using dissected individual nerve fiber examination. He found that there was a decrease in the nerve conduction velocity of the sciatic nerve of the guinea pigs only which had segmental demyelination. Lampert and Schochet[17], Posner[25], and Powell et al.[26] demonstrated demyelination and remyelnation, and proliferation of Schwann cells, resulting in "onion bulbs" in the peripheral nerves of rats by using electron microscopy. To define any potential lesions, if present in the spinal cord and peripheral nerves of cats, the animals should be perfused with appropriate fixative and dissected for individual nerve fiber examination, or electron microscopy should be employed, neither of which has yet been done in cats.

There have been many explanations concerning why one individual develops symptoms of lead toxicity at a certain lead concentration and not another. Reasons for periodic exacerbations and then subsiding symptomology have also been forwarded. Possible theories include:(a) the differential availabilities of diffusable lead versus lead that is stored at more inaccessible sites; (b) recent high dose versus a more chronic exposure to lead; (c) an increase or decrease in the absorption or reabsorption of lead from the gastrointestinal tract; (d) physiologic "trigger" mechanisms such as parathyroid, thyroid, or calcium effects that could mobilize stored lead, particularly in the bone.[25]

The lethal dose of lead for the dog occurs over the range of 10 to 25gm/kg body weight(Volker, 1950:cited by Bloom[3]). From our study, the lethal dose appeared to range from 60 to 150gm/kg body weight, where the blood lead concentrations were 110 ~280μg/100mℓ. Unfortunately, Volker's dog blood lead data was not directly available for us to examine further. In comparison with dogs, cats may be more tolerant to lead intoxication. This could be due to a difference of the amount absorbed into the blood stream through the gastrointestinal tract. It is assumend that most of the lead acetate was excreted in the feces, or was not absorbed, by the reflex projectile vomiting in some of the high dose cats.

A reliable diagnosis of lead poisoning in cats can be made utilizing blood lead, ZPP, and ALAD, and tissue lead analysis. In our study, hair lead values were fairy consistent with blood lead and could be of diagnostic help when other samples are unavailable or lead exposure was previous to the time of sampling.

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고양이의 납중독에 관한 실험적 연구

1. 임상증상 및 실험실적 평가

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초 록

고양이에서 납의 중독량을 결정하고 행동과 임상병리학적인 변화를 밝히고, 납 투여용량과 혈중 납농도와의 관계를 규명하기 위하여 42마리의 고양이를 대상으로 체중에 따라 0(대조군), 10, 100(저용량), 1000, 2000, 4000(고용량) ppm의 lead acetate를 경구적으로 투여하여 납독성을 평가하였다.

고투여용량(1000, 2000, 4000ppm)을 투여한 어떤 고양이는 분출성 구토, 활동항진 그리고 발작을 보였다. 모든 실험군에서 성장율은 변화가 없었다. 고양이의 정상 혈중 납농도는 사람, 개 그리고 소의 혈중 납농도 보다 낮았다. 고양이에서 준임상형 납중독의 혈중 납농도는 3~20μg/100mℓ이었고, 임상형 납중독의 혈중 납농도는 20~120μg/100mℓ이었다. Zinc protoporphyrin 농도는 납 투여용량에 비례하였으며 50μg/100mℓ 이상으로 ZPP 농도가 유의성 있게 증가하는 경우는 임상형의 납중독을 의미하였다. Aminolevulinic acid dehydratase는 모든 납 투여용량에 역비례 관계를 보였고, 고양이의 납노출에 대한 유용한 진단적 지표로 나타났다. 오줌의 aminolevulinic acid 농도는 대개 납투여용량에 따라서 증가하지만 측정치는 개체에 따라 다양하였다. 피모의 납농도는 납투여용량에 따라 비례적으로 증가하였다. 최소한 고용량에서 납은 PMN 세포와 단핵구의 화학주성을 억제하는 것으로 나타났다. 헤모글로빈, 적혈구, 백혈구, 호중구, 임파구, 단핵구 그리고 호산구에서는 투여용량에 대한 반응이 일정하게 관찰되지 않았고 또한 총단백, 혈장단백, BUN 그리고 ALT치에서의 용량에 따른 일정한 변화가 없었다. 망상적혈구수는 대부분의 납투여용량 수준에서 유의성 있게 증가하지 않았기 때문에 고양이 납중독의 진단적 가치는 거의 없었다. 신경전달속도의 유의성 있는 변화가 없는 것으로 보아 납섭취로 인한 帶狀性 脫髓(Segmental demyelination)는 없는 것으로 생각된다. 고양이의 치사량은 체중 kg당 60~150gm이었다. 납중독 진단에 신뢰할 수 있는 파라미터는 혈중 납농도, ZPP, ALAD 그리고 피모의 납농도이었다.