The effect of oral lead on the resistance of hamsters to *Salmonella typhimurium*

by

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ABSTRACT

This experiment was conducted to investigate the effect of oral lead on the resistance of hamsters to S. typhimurium. One hundred and forty-four female golden syrian hamsters (Mesocricetus auratus) were assigned at random to one of 12 treatment groups with each group replicated three times. This resulted in 36 experimental units, each composed of four animals of approximately the same age and weight. The 12 treatments were composed of three exposure periods and four treatments. The exposure periods were 1, 2, and 3 weeks and the treatments 250 mg/kg lead as lead chloride, 125 mg/kg lead as lead chloride, 90 mg/kg chloride as sodium chloride (the equivalent amount of chloride as in the 250 mg/kg lead treatment) and an equal volume of distilled, ion-free water. Subsequent to their respective dosing all animals were challenged with a predetermined LD_{50} dose of S. typhimurium by intraperitoneal injection and observed for mortality during the subsequent 14 day period.

The cumulative mortality after challenge with the LD_{50} dose of S. typhimurium was not significantly different between any of the lead exposed and control groups. There was a highly significant depression in the Δ-aminolevulinic acid dehydratase (ALAD) activities and highly significant increases in whole blood, liver and kidney lead levels in the exposed versus control animals. A small but significant depression in hemoglobin concentrations was noted in the lead exposed animals. There were no statistically significant differences in the times of death, total serum proteins, serum albumin, $alpha_1$ globulin, $alpha_2$ globulin, beta globulin, gamma globulin, body weights, body weight gains, white blood cell counts, differential white blood cell counts, red blood cell counts and hematocrit in the lead exposed versus control animals.

INTRODUCTION

Lead as a health hazard is not, as might be expected, unique to modern man. As long ago as the time of Dioscorides the symptoms of overt lead poisoning were described (Kremers and Urdang, 1940). Although the causes of lead poisoning have been well examined, lead still presents a significant hazard to man and animals. While children in dilapidated urban slums and domestic livestock are the most noted victims of lead intoxication, it is becoming increasingly evident that the general population is also subject to some lead exposure (Chisolm, 1971; Buck, 1970).

The consensus of opinion regarding the acceptable blood lead levels in individuals has changed quite rapidly in recent history. During the first half of the twentieth century it was not uncommon for workers in lead related industries to be allowed to continue working until their blood lead burden exceeded 80 μg/100 ml whole blood (Kehoe, 1966). Currently researchers have suggested levels of safety below 20 μg/100 ml based on the effect of lead on two enzymes important in hemoglobin synthesis, i.e., hemoglobin synthetase and Δ-aminolevulinic acid dehydratase (ALAD) (Chisolm, 1971; Warren, 1974). Lead may be analogous to radiation but with a longer history of learning by experience. As the biological effects of radiation became clearly defined, the maximum permissable exposure levels were lowered until finally it was realized that there was no lower threshold limit for the effects of radiation (Hicks, 1972). A recent study of individuals in 23 cities in the United States revealed little variance in blood lead levels. The mean blood lead level for individuals in the 23 cities was found to be 18.2 μg/100 ml whole

blood (McLaughlin et al., 1973). In light of what has been learned about radiation it seems imperative that this burden be assessed in terms of the biological effects of lead.

HISTORICAL

The history of lead use follows basically two paths, first, the industrial use where the diversity of the metal was exhibited in the form of tools, cooking utensils, lead shot and other applications and second, the medicinal uses of lead in ointments, pain remedies and elixers. In industry the elemental form of lead was largely employed while practitioners of medicine used lead salts in their preparations.

Lead was looked upon as a truly marvelous metal; it was easily formed and virtually indestructable. Because of its great malleability lead found its way into numerous domestic goods. Among the earliest recorded uses of lead in the household was the use of lead in such items as kettles, buckets, rain gutters, flashings, downspouts and lead lined cisterns (McCord, 1954a). Since lead had been known to resist corrosion it seems quite logical that lead would be used to transport water. So it was, that lead became the principle element in metal pipes used to carry water to residences (Ziegfeld, 1964).

In the 1800's the cosmetics industry began to take advantage of the deep and long lasting colors afforded to their products by the use of lead salts as coloring agents. Likewise the paint industry became aware of the durability of paints using lead pigments. Although the use of lead in paints is probably the most widely known of its industrial uses, other industries far surpass the paint industry in annual tonnage used. Most notably are the storage battery and the petroleum products industries (Ziegfeld, 1964; McCord, 1954b).

Until the early 1800's there was no occupational exposure to lead in the United States. At this time the first American manufacturing plants which used lead opened their doors. From this time, until quite recently, the annual tonnage of lead used in the United States has been on the increase. The lead mining industry increased and as more lead became available the smelting and refining industries also soared. It was estimated that in 1962 about 40% of the lead mined in the United States eventually found its way into chemical compounds containing lead while the remaining 60% reached the American consumer largely in the metallic state. This attests to the utility of the metal called lead (Ziegfeld, 1964).

The propriatory properties of lead have been known throughout recorded history. The use of lead plasters can be found in very early history and the adverse effects of lead and lead compounds may be found as early as the time of Dioscorides (Kremers and Urdang, 1940). Since the early authors described the symptoms of lead colic and the unthriftiness in both animals and men who had ingested lead, the thrust of research has been to examine these symptoms and evaluate the causes. Listed below are some of the more important sources of exposure to lead:

Inhalation exposure	Oral exposure	Topical exposure
Vehicle exhaust, fumes from smelting, oil and gasoline refining, paint and varnish production, ceramics manufacture, and insecticides	Ceramics and earthenware pottery, food contamination from fumes and packaging, crop insecticides, paint coloring and drying agents, drinking water contamination	Occupation related - painting, construction, smelting, refining; Nonoccupation related - vehicle exhaust

Research into the magnitude of lead exposure and the possible consequences of that exposure cover many years and innumerable volumes. Some

of the more common sources of lead exposure and the most significant effects of lead on the metabolic processes will be reviewed.

At the lower end of the food chain cereal grains grown in acid soils and especially grains grown in more highly industrialized areas have shown a tendency to retain higher levels of lead than similar grains grown in more neutral soils and in areas of less industrialization. Levels as high as 0.3 to 10.4 parts per million (ppm) have been found in cereal grains grown in highly industrialized areas (Najdenov, 1965). When products are examined for lead content and classified as a source of carbohydrates, fat or protein it has been found that the lead content of carbohydrate foods tends to be higher. Furthermore, among carbohydrates the tendency is toward a positive correlation between the carbohydrate content and the lead content of the food. What this means is that foods with the highest carbohydrate content were found to have the highest levels of lead (Sokalova and Yatsyuk, 1963). Sometimes the way foods are prepared and even the way they are packaged influences the amount of lead ingested. It has been well documented that foods cooked in tinned steel frying pans can contribute a significant amount to the daily intake of lead. This is particularly true if the foods have a naturally low pH or if they are prepared in an acidic medium (Meadows, 1963).

Earthenware glazes, in general, have a high lead content because the lead free glazes are more costly and require special heating and processing. As a result the consumption of foods prepared in earthenware colored with lead containing glazes can present a significant health hazard. Investigations into earthenware glazes as a source of lead ingestion have invariably found this to be a significant source of chronic

exposure to lead. Recently chronic lead poisoning was diagnosed in a physician. He had no apparent occupational source of lead exposure and since no one else in his household showed signs of excessive lead burden, the possibility of contamination through cooking utensils was discounted. Further investigation into his habits revealed that each evening before retiring, the physician indulged in a leisurely cola-flavored soda in his favorite mug. The mug was a gift from his son who had made it in his ceramics class. The mug was, of course, covered with a lead containing glaze. This attests to both the insidious nature of lead poisoning and to the almost inconceivable complexity of the sources of exposure to lead for man and animals (Chisolm, 1971).

Another logical question for researchers to ask is how much lead is there normally in the environment and how much is due to man made sources of pollution? If one considers the task involved here, it becomes obvious that had this question been asked 100 or 200 years ago, answers would be somewhat easier to obtain. Researchers have examined the dust contents of both urban and rural domiciles and speculated that the difference in the lead content of the dust was a measure of how much lead was being contributed to the environment from man made sources. Others have examined the lead content of trees adjacent to modern highways and compared the lead content of the leaves facing the highway with those facing away from the source of lead exposure. The results were then compared with lead levels in vegetation at various distances from the highway. It was concluded that significant amounts of lead were being retained in the leaves facing the highway and the highway presented progressively less of a hazard as

the distance from the highway increased (Vostal et al., 1974). The magnitude of the normal daily exposure to lead in the air for someone living in an urban area has been calculated as about 1 μg of lead per cubic meter of air. This would result in the absorption of about 10 μg of lead per day for the average individual in an urban area. For the average individual in a rural area the normal daily intake of lead from the air would be about ten times less (0.1 $\mu g/M^3$) and the daily absorption of inhaled lead would be about 1.5 μg of lead per day. A reasonable estimate of the total normal daily absorption of lead from food sources is in the range of 15-40 μg of lead per day. This, coupled with the above figures for inhaled lead, means that an urban individual may absorb 25-50 μg of lead per day while for the rural individual may absorb 17-42 μg of lead per day.

What does this mean in terms of the burden placed on the normal function of our body systems? It has been shown that if an individual absorbs 50 μg of lead per day the whole blood lead level (Warren, 1974) tends to be about 20 μg per 100 ml of whole blood. It has also been shown that, at least for the Δ-aminolevulinic acid dehydratase enzyme, any level of lead present at the site of action of this enzyme will inhibit its activity to some degree (Chisolm, 1971).

The body, under normal circumstances, is likely to prevent any significant inactivation of those systems which are affected by lead. Not only will more than 90% of the lead ingested never be absorbed under normal circumstances but some estimates suggest that as much as half of the lead that is absorbed finds its way into the urine and feces to be evacuated. The remaining lead, that which has not been excreted, will be shunted to the bone matrix where it is held in complex to prevent any

adverse metabolic effects due to free lead. Any other lead will be found in the soft tissues of the body (especially liver and kidney). Thus under normal conditions the body is adequately protected from the deleterious effects of lead (Kehoe, 1964).

It has been stated that under normal conditions the body can cope with the normal daily burden of lead from the ambient environment. But what are the consequences if the environment changes as it has since the industrial revolution? What then are the consequences of excessive lead ingestion and absorption?

Lead can react with certain functional groups which are called ligands and the importance of this interaction of lead with ligands is seen when it is realized that all biological systems are under enzymatic control and all enzymes have ligands. Why then does lead only inhibit certain enzymes? This is explained by the shape of the enzyme; some have ligands which are protected by nonreactive groups. Other enzymes are intracellular and the cell membrane ties up the lead preventing its action on the enzyme (Passow et al., 1961; Rothstein, 1959). Under experimental conditions lead has been shown to significantly depress the activity of Δ-aminolevulinic acid dehydratase (ALAD), an enzyme associated with the production of hemoglobin. This depression occurs, in vitro, with very low lead levels at the sight of action of ALAD. This indicates that lead, like radiation, has no lower limit of effect (Chisolm, 1971). Numerous other enzymes have been examined for lead affects but only ALAD and heme synthetase have been shown to be significantly affected by lead (Carson et al., 1973; Chisolm, 1971; Rogers et al., 1971).

Since early in the 1800's the pallor of post-mortem tissues and reduced red blood cell count have been recognized and associated with lead poisoning. Lead induced anemias have been reported to be caused by the reduced hemoglobin concentration resulting from the inhibition of the two enzymes mentioned above. Although anemias have been reported in cases of lead poisoning in humans and demonstrated experimentally in some animals, lead poisoning in domestic animals has seldom been associated with coexistent anemia (Buck, 1970). Erythrocytes in lead poisoned individuals were shown to have a very high rate of potassium turnover and a coexistent net loss of potassium. This does not appear to make them more fragile than normal erythrocytes but they are reported to have a shorter life span (De Bruin, 1971; Rothstein, 1959; Waldron, 1966).

Serum proteins were observed to undergo electrophoretic pattern changes in cases of severe lead poisoning and there was a significant decrease in the albumin:globulin ratio, with the largest increase in the beta globulin fraction. In addition to the effect on hemoglobin synthesis and the altered electrophoretic pattern of serum proteins, lead poisoning has caused severe renal dysfunction and the loss of metabolically important amino acids (De Bruin, 1971).

In recent years it has been suggested that lead may cause alterations in the normal resistance of certain species to infectious agents. It appears that lead may act in some way to depress the defense mechanisms of the animal, thus facilitating the disease process. It has been reported that lead exposure altered the normal ratio of albumin to globulin in rabbits and reduced the normal antibody response to vaccination. The

antibacterial properties of rabbit's blood were also abolished in the presence of lead (Fonzi et al., 1967a; Fonzi et al., 1967b).

The death of a young child was investigated and attributed to a bacterial pathogen but it was suggested that the pathogen would not have been able to cause the death of the child unless the child's normal defense mechanisms had been altered by lead (Williams et al., 1954). Other toxicants including sulfur dioxide, carbon dust, polychlorinated biphenyls and cigarette smoke have been shown to reduce the normal antibody response (Friend and Trainer, 1970).

Chicks exposed to lead acetate and endotoxin from Escherichia coli succumbed at a significantly greater rate than controls (Truscott, 1970) and ducks exposed to polychlorinated biphenyls and duck hepatitis virus had a significantly higher mortality than controls (Friend and Trainer, 1970). Mice administered lead acetate intraperitoneally (IP), which did not show overt signs of toxicosis, had significantly higher mortality than nonexposed animals following exposure to a pathogenic strain of S. typhimurium (Hemphill, 1971).

These reports clearly raise questions of whether or not lead and other environmental pollutants have twofold effects: (a) direct toxic effects such as enzyme inactivation; and (b) indirect effects on the resistance mechanisms to pathogenic microorganisms.

EXPERIMENTAL PROCEDURES

Hamsters

One hundred and forty-four apparently healthy, female golden syrian hamsters (Mesocricetus auratus), specified to be free from previous exposure to Salmonella typhimurium, were obtained from ARS/Sprague Dawley, Madison, Wisconsin. All of the hamsters were 3-4 weeks old and ranged in weight from 25-30 grams upon arrival. Upon receipt, the hamsters were assigned at random to one of 36 opaque polycarbonate cages (14"x8.5"x5") which provided approximately 0.1 square feet of floor space per animal and were 0.4 feet high. Access to feed and water was provided through a stainless steel slotted cage top. The hamsters were given a commercial laboratory ration[1] and water ad libitum. Adequate ventilation was maintained and the ambient temperature ranged between 70-75°F. The room was isolated from outside sources of light and a constant 10-hour light, 14-hour dark cycle was maintained throughout the entire experimental period with the use of standard fluorescent laboratory lighting. Each animal was handled daily beginning on day one of the experimental period. Day one corresponded to 1 week after the actual receipt of the animals. The first week represented the acclimation period.

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[1]Wayne Lab-Blox Shorts, Allied Mills, Inc., Chicago, Ill. 60606.

Lead Compounds

Reagent grade lead chloride was obtained from a commercial source.[1] Lead solutions in ion-free water were prepared once each week to adjust for the weight gain (or loss) during the experiment. The lead solutions were prepared to contain the respective dose of lead in a volume not to exceed 2-3% of the average body weight of the hamsters.

Determination of an LD_{50} Dose of Salmonella typhimurium

An additional 48 female hamsters, 6-7 weeks of age, were randomly assigned to 12 cages, four animals per cage. These animals were allowed to acclimate for 1 week at which time three groups of four cages were randomly selected and assigned a number from 1 through 4. Each animal in the cages marked 1 was given 0.5 ml of a 1:10 dilution of a 4 hour culture of Salmonella typhimurium. This organism was characterized and isolated from porcine tissues submitted to the Veterinary Diagnostic Laboratory, Ames, Iowa, culture #6337. Likewise, each animal in cages 2, 3 and 4 was given 1:100, 1:1000 and 1:10,000 dilutions, respectively, of the same 4 hour culture. These animals were challenged and observed for 14 days. Times of death and overall mortality in each replicate were recorded. A quantitative plate count was conducted on the same 4 hour culture and it was determined that the concentration of organisms was approximately 2×10^9 bacteria/ml. After assimilation of the mortality data from the three replicates, the LD_{50} dose was calculated using the method of Reed

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[1] J. T. Baker Chemical Co., Phillipsburg, N.J.

and Muench (1938). It was determined that the LD_{50} dose of Salmonella typhimurium in 7-8 week old hamsters was 0.5 ml of a 1:251 dilution of a 4 hour culture. This was approximately $1x10^{-2.4}$ dilution and contained $4x10^6$ organisms. A second quantitative plate count was conducted 24 hours prior to the introduction of the S. typhimurium into the experimental design described below. This revealed that the concentration of the organism had remained the same as when it was used to determine the LD_{50} dose.

Experimental Design

The experiment consisted of a 1 week acclimation period for all 144 animals followed by 1, 2 or 3 weeks of exposure to the following treatments: 250 mg/kg lead as chloride; 125 mg/kg lead as chloride; 90 mg/kg chloride as sodium chloride (the equivalent weight of chloride in the 250 mg/kg lead treatment); or an equal volume of distilled ion-free water. The final stage of the experiment involved challenging the lead-exposed and control groups with an LD_{50} dose of S. typhimurium followed by a 14 day observation period.

In order to accommodate three different lengths of lead exposure and to eliminate possible cross contamination before the end of the lead exposure regimen, the following design was developed. On day one of the experimental period all 144 animals were individually weighed and their weights were recorded. The overall average weight was calculated to be 61.9 g and the lead doses were determined according to this weight. Each day the order of dosing was randomized by cage and treatment group to avoid systematic errors in the results. During the week comprised of

days 1-7, 48 animals received actual lead or control doses while the remaining 96 animals were handled to the same degree but received no oral treatment. During the week comprised of days 8-14, 96 animals received the appropriate oral exposure to the above treatments while the remaining 48 animals received the same degree of handling. On days 15-21 all 144 animals received the appropriate oral exposure by gastric tube. Intubation was achieved by the use of an 18-gauge curved ball tipped feeding needle (2 1/4 mm ball) attached to sterile disposable 3 ml syringes. Intubation was conducted at approximately 4 hours before the end of the light cycle.

On day 22 of the experiment, approximately 24 hours after the final lead dose, all 144 hamsters were challenged with a previously determined LD_{50} dose of Salmonella typhimurium. This dose was contained in a 0.5 ml volume of sterile normal saline solution and was administered intraperitoneally. By this design, the infectious agent was introduced into the experiment only once, thereby eliminating any possible crossover infections before the end of the lead exposure period and eliminating any treatment differences due to variation in age of the S. typhimurium cultures. The animals were observed for 14 days subsequent to S. typhimurium challenge for mortality and time of death.

Blood Collection

Each animal was anesthetized by being placed in a wide-mouthed glass jar, partly filled with ether soaked cotton. Weekly blood samples were taken from an orbital sinus of each animal using a 100 ul capillary tube. The samples were taken 24 hours before the first dosing and 24 hours after

the previous day's dosing for each subsequent sample. They were pooled into heparinized lead-free vacutainers[1] for whole blood lead analysis (Hessel, 1968), into EDTA vacutainers for complete blood counts and into additive-free tubes for serum separation. Approximately 1 ml of blood was taken from each animal during the experiment on days 0, 7, 14 and 21. Each of the following determinations was made on each pooled blood sample: (1) red blood cell count; (2) hemoglobin concentration; (3) hematocrit; (4) white blood cell count; (5) differential white blood cell count; (6) ALAD (Burch and Siegel, 1971); (7) whole blood lead concentrations (Hessel, 1968); (8) total serum proteins; and (9) serum protein fraction percentages. Standard clinical chemistry methods were employed for all procedures unless otherwise specified.

Serum Protein Determinations

The blood samples collected in additive-free vacutainers were allowed to clot at room temperature for 1 hour and stored in a refrigerator at 4°C overnight. The samples were centrifuged at 1500 rpm for about 15 minutes to settle the clot. The serum was frozen at -20°C in 3.7 ml vials until the serum protein determinations were made.

Total serum protein was determined using a refractometer.[2] The instrument was held up to a source of light after filling the sample chamber with approximately 0.05 ml of thawed pooled serum. The total serum proteins were read on the internal scale in g/100 ml.

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[1] Becton-Dickinson and Co., Rutherford, N.J.

[2] T.S. meter, American Optical Co., Buffalo, N.Y.

Fractionation of the serum proteins was achieved on cellulose acetate membranes[1] in a microzone chamber[2] using tris-barbital buffer[3] at pH 8.8 and ionic strength 0.05. Each cellulose acetate membrane accommodated six 0.25 µl samples and the fractionation chamber accommodated three cellulose acetate membranes. Thus, 18 serum samples were separated simultaneously. The proteins were separated at 250 V for 40 minutes and stained with Ponceau S stain[4] following separation.

A scanning photoelectric densitometer[5] was employed to quantitate the relative amounts of each protein fraction based on the amount of light absorbed from the stained protein bands. An integrated density curve was plotted and the optimal density was measured at 600 nm. The density peaks were separated by drawing vertical lines through the lowest point in each of the fractions and the relative percentage of each of the fractions was calculated.

Tissue Samples

Necropsy examinations were performed on all animals that died following exposure to S. typhimurium, and on all surviving animals after they were euthanized. The liver and kidneys were taken for lead analysis.

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[1]Brinkman Sartorius, Brinkman Instruments, Westbury, N.Y.

[2]Beckman Instruments, Fullerton, California.

[3]Gilman Instrument Company, Ann Arbor, Mich.

[4]Buchler Instruments, Fort Lee, N.J.

[5]Beckman Instruments, Fullerton, California.

A representative sample of each animal's liver and both kidneys were dryed in an oven overnight, then ashed in a muffle oven at about 450°C. The lead in these samples was extracted into 2N hydrochloric acid and aspirated in a Perkin-Elmer Model 303 atomic absorbtion spectrophotometer. A standard curve was constructed and the amount of lead in each sample was calculated from this curve.

Analysis of Data

All data collected were analyzed using the Statistical Analysis System (Service, 1972) at the Iowa State University Computation Center. An analysis of variance was conducted on the data to examine and test the data for treatment differences. A cross products analysis was conducted on the data to examine the relationships among the data collected. A regression analysis was conducted on the data to test the significance of the linearity of the data.

RESULTS

The tests of significance for the lead exposed and control animals are shown in Table 1. The following parameters were shown to differ significantly among the treatment groups: blood lead, significant at P=.01; ALAD, significant at P=.01; hemoglobin concentration, significant at P=.01; and hematocrit, significant at P=.03. There were no significant treatment differences among the four groups for each of the following parameters: red blood count, white blood count, differential white blood count, body weights, body weight gains, times of death, percent mortality, total proteins, albumin (%), alpha$_1$-globulin (%), alpha$_2$-globulin (%), beta-globulin (%) and gamma-globulin (%).

Blood Lead

Before the initiation of the experimental protocol, the overall mean blood lead level was 10.8 µg/100 ml; by the end of the experiment the overall mean blood lead level had risen to 59.5 µg/100 ml. The overall mean blood lead levels were calculated using all of the experimental units regardless of treatment. The overall blood lead levels progressed from 10.8 µg/100 ml in week 1 to 22.6 µg/100 ml in week 2. By the end of the third week the blood lead level had risen to 40.6 µg/100 ml and immediately before challenge with Salmonella typhimurium was 59.5 µg/100 ml.

The mean blood lead levels by treatment are given in Table 2. At the end of lead exposure (immediately before challenge with Salmonella typhimurium), the blood lead levels were 18 µg/100 ml in the H_2O controls,

Table 1. Test of treatment differences

Parameter	MST[a]	MSE[b]	F[c]	S or N.S.[d]
Blood lead	13.85	0.24	57.71	S-.01
Δ-aminolevulinc acid dehydratase	127611	1826	69.88	S-.01
Red blood count	0.816	2.11	0.39	N.S.
Hemoglobin	11.12	1.53	7.30	S-.01
White blood count	1572	1756	0.90	N.S.
Neutrophile count	37.63	256	0.15	N.S.
Bands count	106.6	42	0.5	N.S.
Lymphocyte count	45.7	267	0.17	N.S.
Monocyte count	4.92	3.52	1.39	N.S.
Eosinophil count	2.32	1.78	1.31	N.S.
Hematocrit	48.25	13.72	3.52	S-.03
Body weight	97.9	239.2	0.41	N.S.
Gain	43.5	20.2	2.15	N.S.
Time of death	335.2	3911.1	0.09	N.S.
Percent mortality	883.1	3247.9	0.27	N.S.
Total proteins	0.33	0.19	1.74	N.S.
Albumin (%)	68.88	90.14	0.76	N.S.
Globulins				
$Alpha_1$ (%)	10.17	85.80	0.12	N.S.
$Alpha_2$ (%)	34.33	48.97	0.70	N.S.
Beta (%)	238.80	172.23	1.39	N.S.
Gamma (%)	19.58	44.72	0.44	N.S.

[a]MST = mean square treatment.

[b]MSE = mean square error.

[c]F = variance ratio = $\frac{MST}{MSE}$ equivalent to a Student's t test of significance.

[d]S or N.S. = significant or no significant difference.

15 µg/100 ml in the NaCl controls, 74 µg/100 ml in the 125 mg/kg lead and 131 µg/100 ml in the 250 mg/kg lead exposed animals (Table 2, Figure 3).

An analysis of variance for the blood lead data showed significant differences among the treatment groups (Table 3). The two control groups were not significantly different, while the average of the two controls was significantly lower than the average of the two lead treated groups (P=.01) (Figure 1). The initial blood lead levels were essentially the same in the 125 mg/kg exposure group regardless of the age at which lead exposure was initiated (Figure 1). After 1 week of lead exposure the animals of age 5, 6, and 7 weeks had mean blood lead levels of 66, 61 and 73 µg/100 ml, respectively. These were not significantly different. The final blood lead levels in the three age subgroups of the 125 mg/kg group did not differ significantly from one another (Table 1, Figure 1).

The three age subgroups in the 250 mg/kg had significantly different blood lead levels after 1 week of lead exposure (Figure 1). Although the 5 and 6 week old animals did not differ significantly in their mean blood lead levels, the 7 week old animals had significantly higher blood lead levels than either the 5 or 6 week old group (Table 1).

The 7 week old animals had a mean blood lead level of 159 µg/100 ml after 1 week exposure while the 5 and 6 week old animals had mean blood lead levels of 118 and 103 µg/100 ml, respectively. These differences between the 7 week old animals and the 5 and 6 week old animals were significant at the 5% and 1% levels of significance, respectively.

The final blood lead levels within the age subgroups in the 250 mg/kg exposure group were significantly different (P=0.05). The 7 week old

Table 2. Blood lead[a] data for control and lead exposed hamsters (µg/100 ml) mean (range)[b]

Age (weeks)	Total weeks of exposure to date	Group I H_2O control	Group II NaCl control	Group III 125 mg/kg Pb	Group IV 250 mg/kg Pb	Overall week mean[c]
5	0	2(0-6)	12(-)[d]	15(12-22)	7(0-21)	
	0	3(0-6)	11(0-20)	20(9-30)	10(0-24)	11
	0	12(0-24)	4(0-12)	15(0-24)	18(0-.42)	
6	0	10(6-12)	10(6-21)	5(0-9)	11(9-15)	
	0	6(3-12)	12(6-18)	12(9-15)	8(6-12)	23
	1	4(0-9)	10(6-16)	61(54-66)	118(105-132)	
7	0	10(0-15)	6(3-18)	1(0-3)	4(0-9)	
	1	2(0-3)	7(3-10)	66(60-69)	103(93-108)	41
	2	0(-)	3(0-8)	72(63-81)	214(120-378)	
8	1	20(9-34)	16(9-36)	73(66-84)	159(117-216)	
	2	22(18-26)	10(9-12)	67(48-87)	118(99-138)	60
	3	12(-)	18(12-24)	83(75-90)	116(105-138	

[a]Each blood lead represents the lead content of a 1 ml sample of pooled blood from each cage of four hamsters.

[b]Mean (range); each mean represents the average of three pooled samples from a total of 12 animals.

[c]Each overall week mean represents the average across all groups.

[d]- = no range, all readings identical.

Table 2. (Continued)

Age (weeks)	Total weeks of exposure to date	Group I H_2O control	Group II NaCl control	Group III 125 mg/kg Pb	Group IV 250 kg/kg Pb	Overall week mean
Mean for week						
5		6	9	17	12	
6		7	11	26	47	
7		4	5	46	107	
8		18	15	74	131	

Figure 1. Whole blood lead levels for control and lead exposed hamsters. TEX = total length of treatment exposure. Grouped by length of exposure.

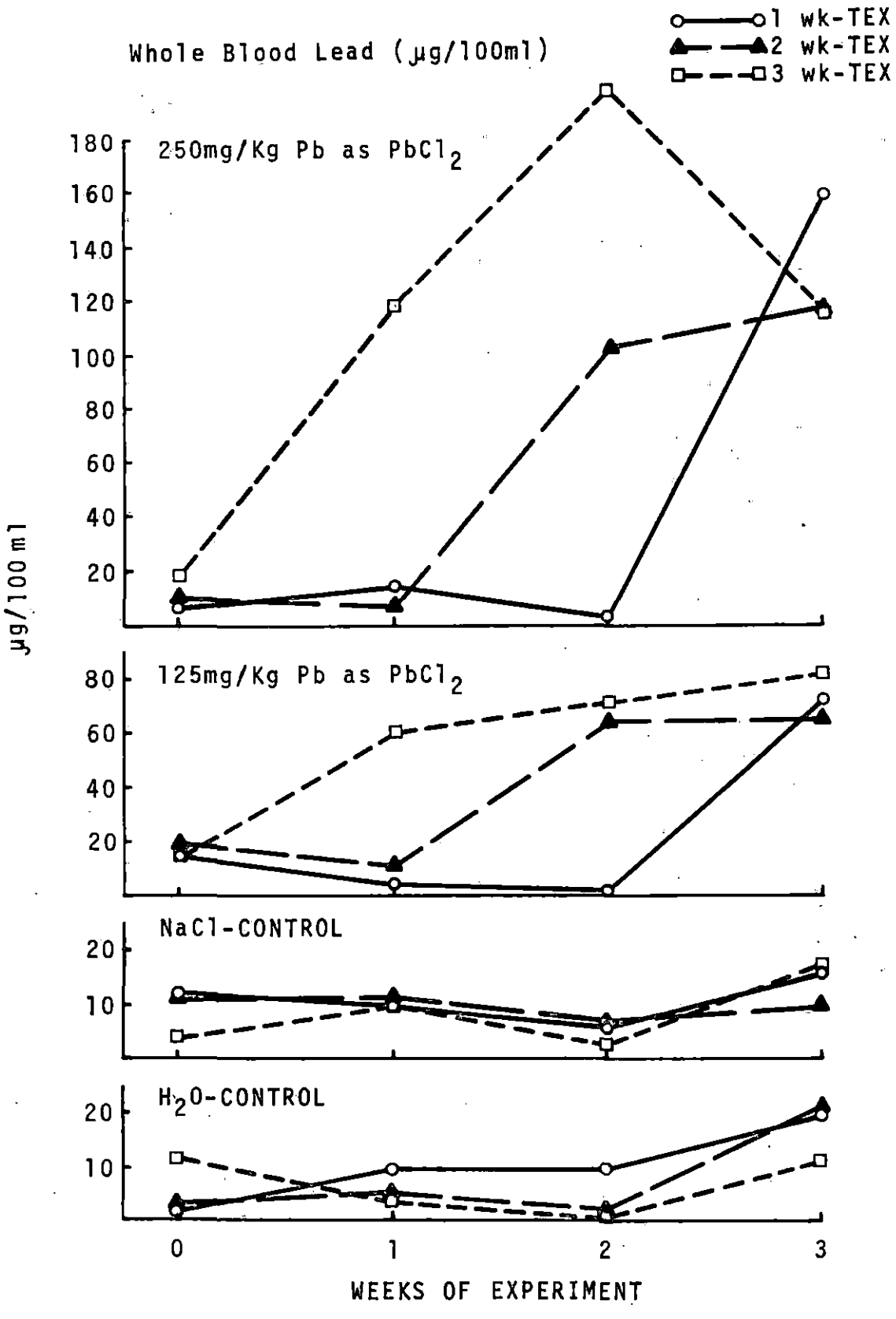

Whole Blood Lead (µg/100ml)
1 wk-TEX
2 wk-TEX
3 wk-TEX
250mg/Kg Pb as PbCl2
125mg/Kg Pb as PbCl2
NaCl-CONTROL
H2O-CONTROL
µg/100ml
180
160
140
120
100
80
60
40
20
10
0
1
2
3
WEEKS OF EXPERIMENT

Table 3. Tests of significance for blood lead data

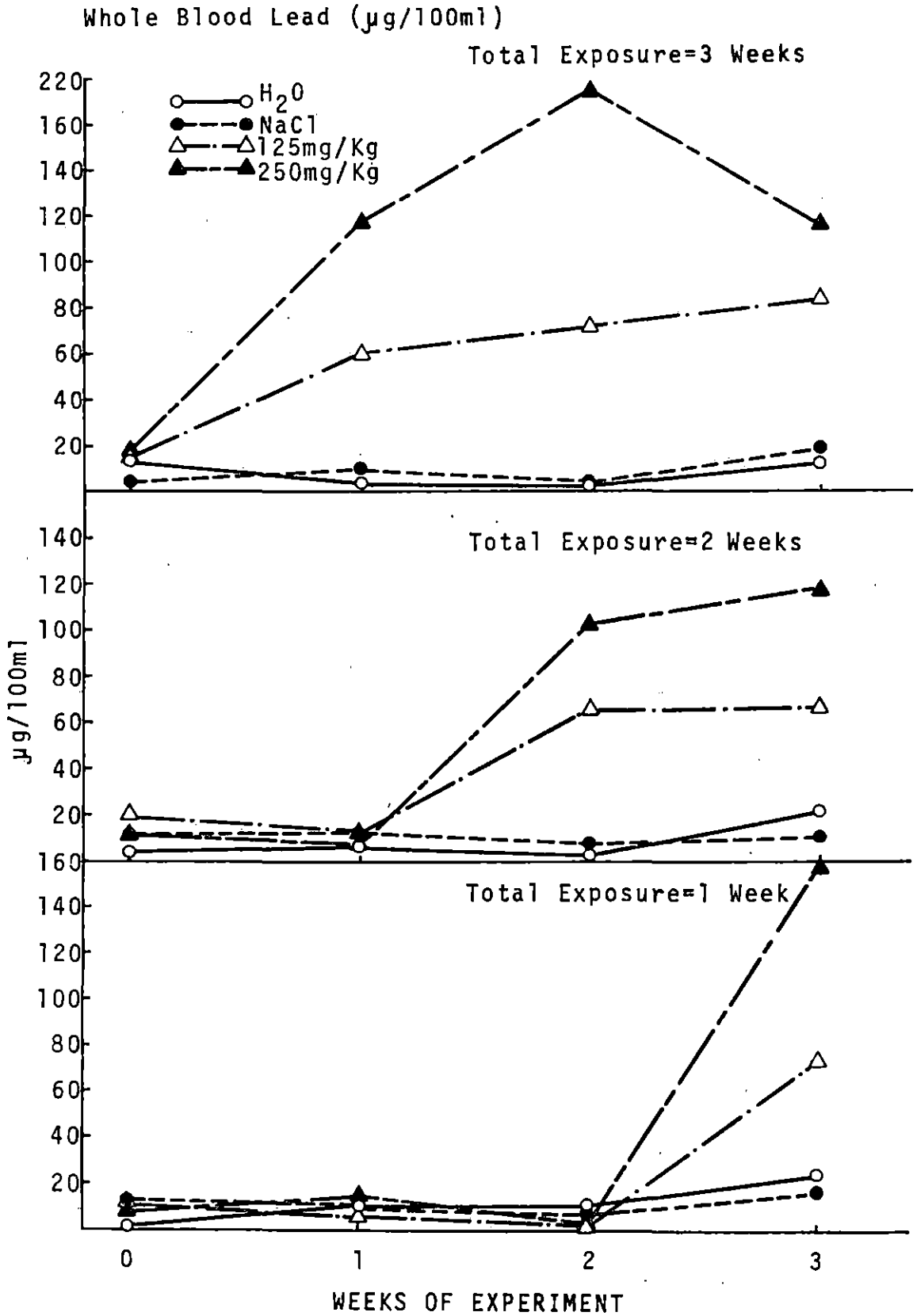
Group	Contrasts	Probability of a larger t
H_2O control (t_1)	$\frac{t_1+t_2}{2}$ vs $\frac{t_3+t_4}{2}$	P<0.01
NaCl control (t_2)	t_1 vs t_2	N.S.[a]
125 mg/kg Pb (t_3)	t_3 vs t_4	P<0.01
250 mg/kg Pb (t_4)		
Total exposure of 1 week (TEX_1)	TEX_1 vs TEX_2	N.S.
Total exposure of 2 weeks (TEX_2)	TEX_2 vs TEX_3	P<0.01
Total exposure of 3 weeks (TEX_3)		
Interactions	$\frac{t_1*TEX_3+t_2*TEX_3}{2}$ vs $\frac{t_3*TEX_3+t_4*TEX_3}{2}$	P<0.01
	t_3*TEX_3 vs t_4*TEX_3	P<0.01

[a]No significant difference.

animals had higher levels than the two groups of younger animals, 116, 118, and 159 µg/100 ml in the 5, 6, and 7 week old animals, respectively (Table 1, Figure 1).

Mean blood lead levels were grouped by total weeks of lead exposure and presented in Figure 2. Overall group blood lead levels by total length of exposure are presented in Figure 3.

Figure 2. Whole blood lead levels for control and lead exposed hamsters, grouped by treatment.


Whole Blood Lead (µg/100ml)
Total Exposure=3 Weeks
H2O
NaCl
125mg/Kg
250mg/Kg
Total Exposure=2 Weeks
Total Exposure=1 Week
µg/100ml
WEEKS OF EXPERIMENT

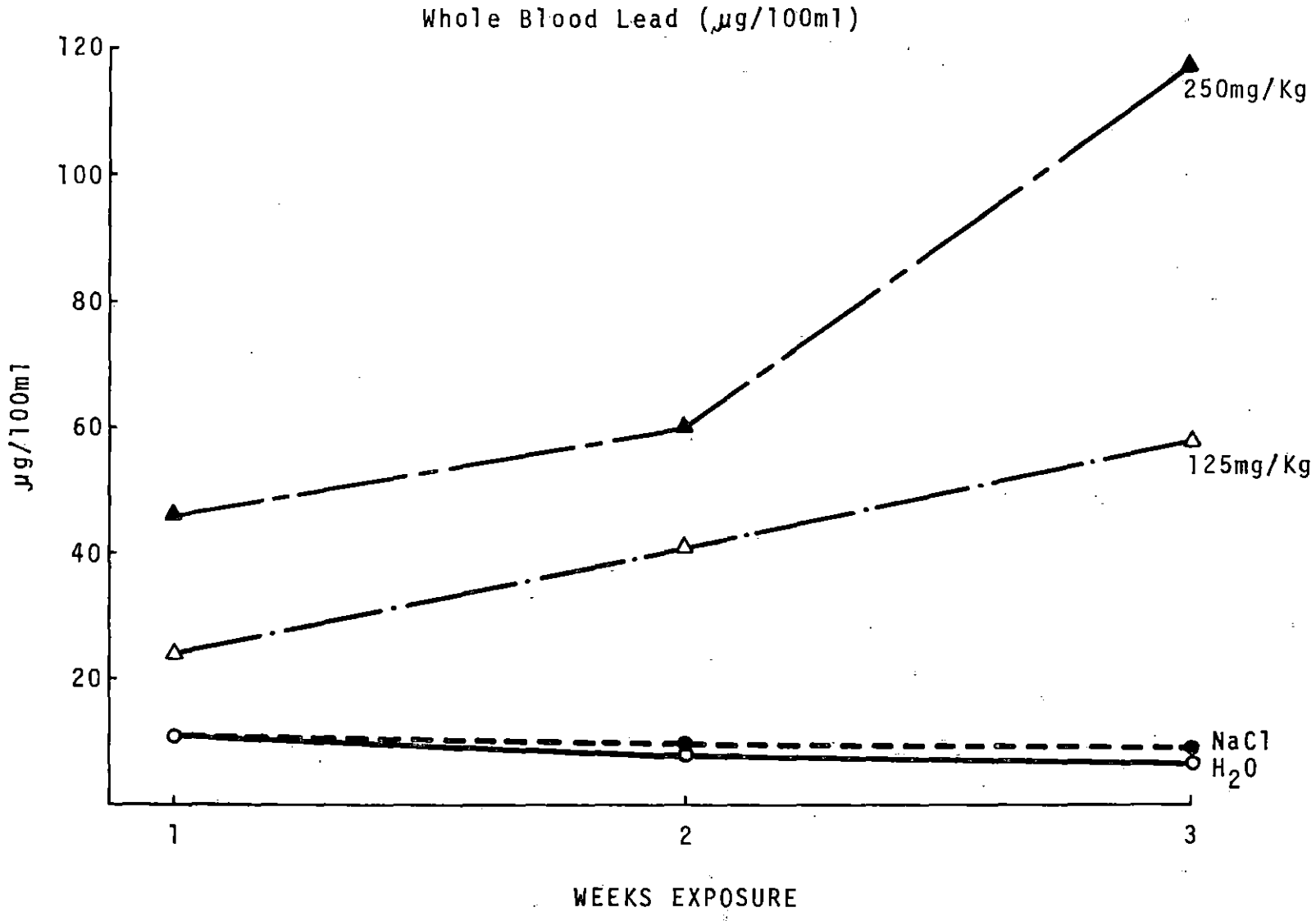


Figure 3. Mean whole blood lead levels by total length of treatment exposure.

Δ-Aminolevulinic Acid Dehydratase Activity

The overall mean ALAD activity before lead exposure was 155 units ranging from 106-244. At the end of lead exposure the overall mean activity in control and exposed groups was 85 units ranging from 10-153 (Figures 4, 5 and 6). Although all of the groups had a decrease in activity, there were significant differences between the lead exposed and control animals.

The controls did not differ significantly from one another. The two lead exposed groups did not differ significantly from one another, but the lead exposed groups had a significant reduction in ALAD activity in comparison with the controls (Table 4).

The progressive inactivation of ALAD can be seen in Figures 4 and 5.

Within the lead treated groups the blood lead levels and the activity of ALAD were negatively correlated. This means, first, that as the blood lead level increases the activity of ALAD decreases and second, this inactivation tends to be linear at the 125 mg/kg exposure, while at the 250 mg/kg exposure the inactivation of the enzyme tended to be curvilinear over the observed range. The correlation between blood lead levels and the level of enzyme activity within the 125 mg/kg Pb treatment group was -0.89 and in the 250 mg/kg Pb treatment group it was -0.80. Thus, in the 125 mg/kg Pb treatment groups 79% (R^2=.79) of the observed variation in the ALAD activity can be explained by the blood lead data. Likewise for the 250 mg/kg Pb treatment 64% (R^2=.64) of the observed variation in enzyme activity can be explained by the presence of high blood lead levels (Steel and Torrie, 1960).

Figure 4. Whole blood Δ-aminolevulinic acid dehydratase activities for control and lead exposed hamsters. TEX = total length of treatment exposure. Grouped by length of exposure.

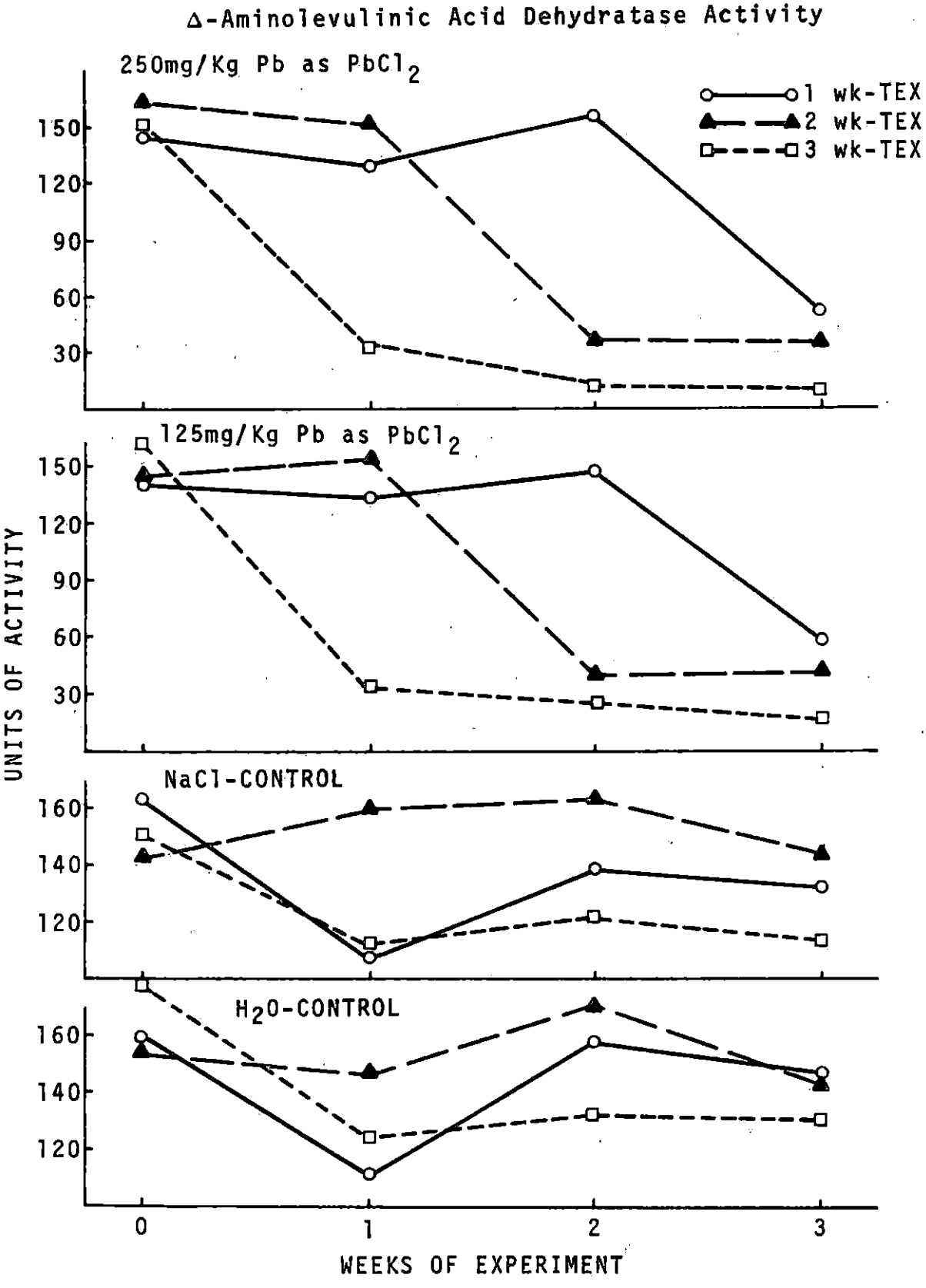

Δ-Aminolevulinic Acid Dehydratase Activity
250mg/Kg Pb as PbCl2
1 wk-TEX
2 wk-TEX
3 wk-TEX
150
120
90
60
30
125mg/Kg Pb as PbCl2
150
120
90
60
30
NaCl-CONTROL
160
140
120
H2O-CONTROL
160
140
120
UNITS OF ACTIVITY
0
1
2
3
WEEKS OF EXPERIMENT

Figure 5. Whole blood Δ-aminolevulinic acid dehydratase activities for control and lead exposed hamsters, grouped by treatment.

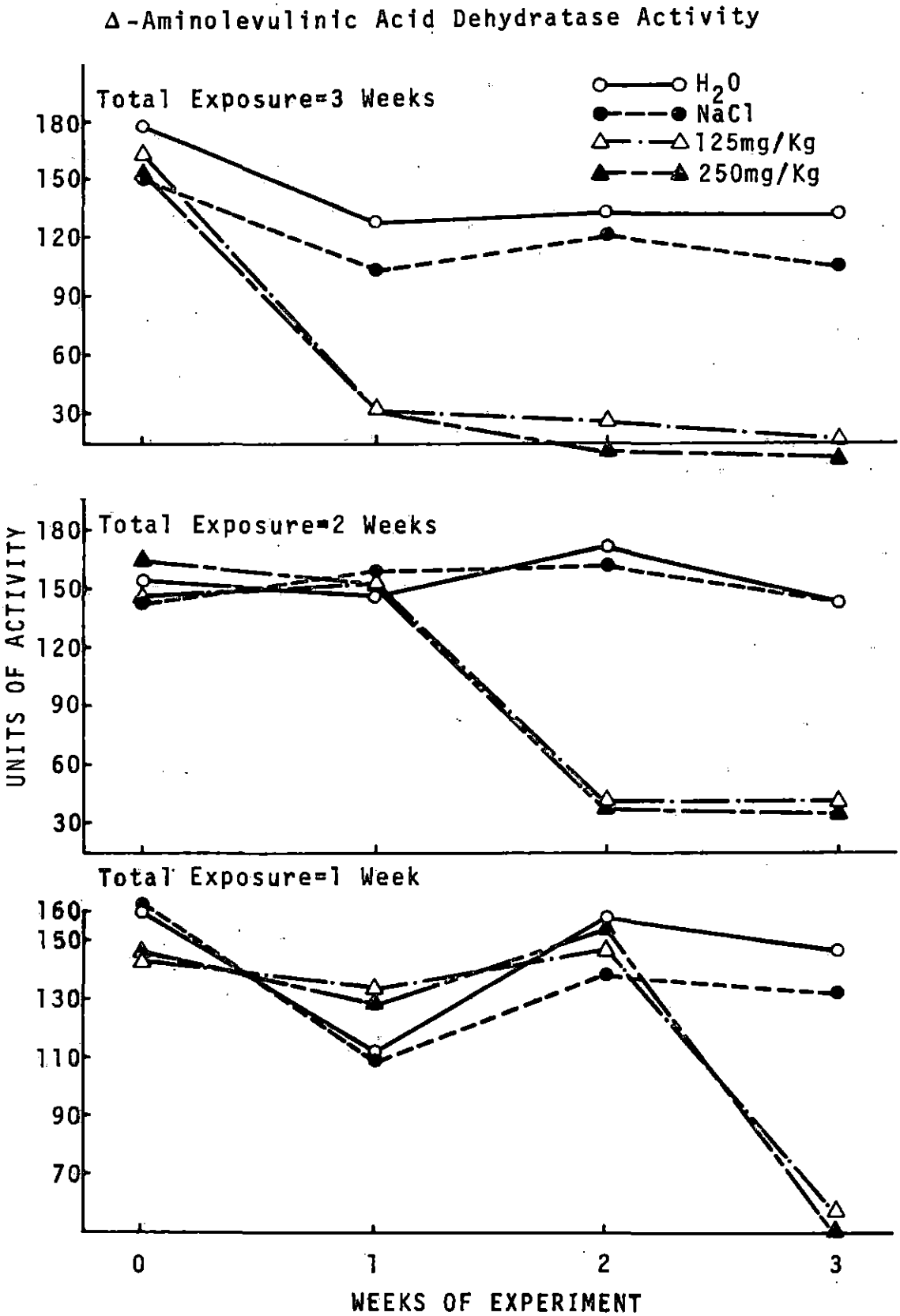

Δ-Aminolevulinic Acid Dehydratase Activity
Total Exposure=3 Weeks
H2O
NaCl
125mg/Kg
250mg/Kg
180
150
120
90
60
30
Total Exposure=2 Weeks
UNITS OF ACTIVITY
180
150
120
90
60
30
Total Exposure=1 Week
160
150
130
110
90
70
0
1
2
3
WEEKS OF EXPERIMENT

Figure 6. Whole blood Δ-aminolevulinic acid dehydratase activities for control and lead exposed hamsters as a function of total length of exposure.

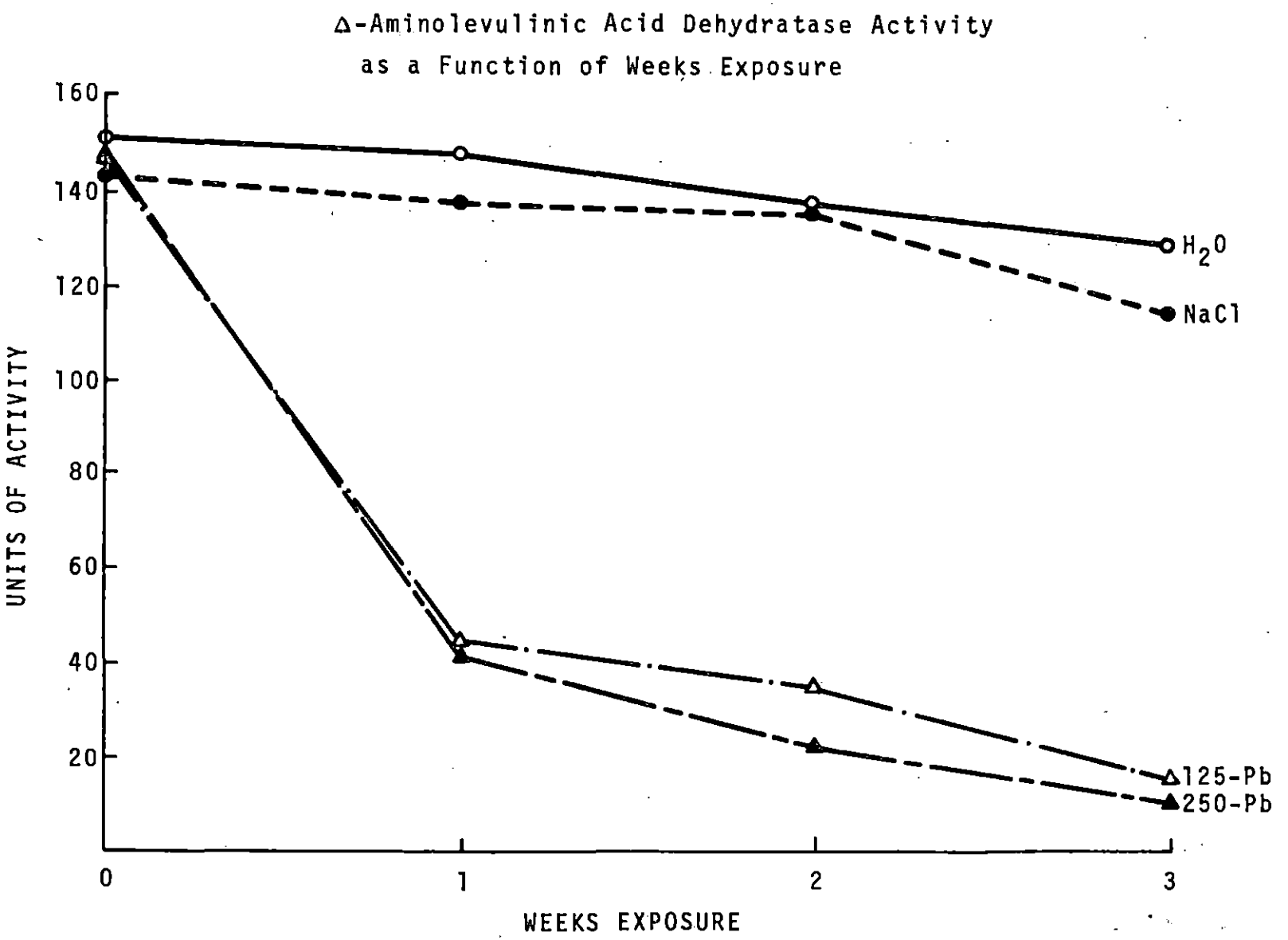

Δ-Aminolevulinic Acid Dehydratase Activity
as a Function of Weeks Exposure
UNITS OF ACTIVITY
160
140
120
100
80
60
40
20
0
1
2
3
WEEKS EXPOSURE
H2O
NaCl
125-Pb
250-Pb

Table 4. Tests of significance for Δ-aminolevulinic acid dehydratase data

Group	Contrasts	Probability of a larger t
H_2O control (t_1)	$\frac{t_1+t_2}{2}$ vs $\frac{t_3+t_4}{2}$	P<0.01
NaCl control (t_2)	t_1 vs t_2	N.S.[a]
125 mg/kg Pb (t_3)	t_3 vs t_4	N.S.
250 mg/kg Pb (t_4)		
Total exposure of 1 week (TEX_1)	TEX_1 vs TEX_2	N.S.
Total exposure of 2 weeks (TEX_2)	TEX_2 vs TEX_3	P<0.01
Total exposure of 3 weeks (TEX_3)	TEX_1 vs TEX_3	P<0.01
Interactions	$\frac{t_1*TEX_3+t_2*TEX_3}{2}$ vs $\frac{t_3*TEX_3+t_4*TEX_3}{2}$	P<0.01
	t_3*TEX_3 vs t_4*TEX_3	N.S.

[a]No significant difference.

The initial rate of enzyme inactivation was not significantly different in the 125 mg/kg and 250 mg/kg exposure groups (Figures 4 and 5). The mean ALAD activity in 125 mg/kg group was 33 units of activity for those animals receiving initial exposure to lead at 5 weeks of age. This was not significantly different from the means of the animals receiving initial lead exposure at 6 and 7 weeks of age (40 and 57 units of activity, respectively). The initial rate of inactivation of ALAD did not

differ significantly among the three age subgroups in the 250 mg/kg group. This is evidenced by the means for ages of 5, 6 and 7 weeks which were 33, 37 and 51 units of activity, respectively (Figure 4 and 5). The control groups did not differ significantly in the levels of enzyme activity (Figure 6).

The mean ALAD activity for the 125 mg/kg group following 1 week of exposure was 120 units, which was significantly higher ($P<.01$) than activities after 2 and 3 weeks of exposure, 95 and 60 units, respectively.

A similar pattern was observed in the 250 mg/kg treatment group. The means for 1, 2 and 3 weeks of exposure were 120, 97 and 52 units of activity, respectively. These means were significantly different ($P<0.01$) (Table 5).

The means for the 125 mg/kg and the 250 mg/kg groups were not significantly different at each of the total lengths of exposure.

Body Weights and Body Weight Gains

The final mean body weights for the H_2O control and the NaCl control were 80.0 and 80.7 grams, respectively. The final mean body weights for the 125 mg/kg and 250 mg/kg groups were 78.9 and 80.1 grams, respectively. These means were not significantly different (Table 6).

These data were subjected to an analysis of variance and the variance ratio was calculated. There were no significant treatment differences among the lead treated and control animals (Figure 7 and 8). There was, however, a trend toward lower body weight as the length of exposure increased (Figure 9). This trend was seen in both control and lead exposed

Table 5. Δ-Aminolevulinic acid dehydrase activities for control and lead treated animals, mean[a] (range)

Age (weeks)	Total weeks of exposure to date	Group I H_2O control	Group II NaCl control	Group III 125 mg/kg Pb	Group IV 250 mg/kg Pb
5	0	160(138-183)	162(106-244)	143(128-155)	145(135-154)
	0	155(131-188)	143(138-149)	145(116-175)	164(140-189)
	0	178(167-193)	151(117-176)	164(160-169)	153(145-160)
6	0	111(102-120)	109(91-125)	133(90-183)	128(101-172)
	0	147(-)	159(137-190)	155(147-172)	153(152-154)
	1	128(102-148)	112(109-116)	33(29-39)	33(24-41)
7	0	158(144-171)	138(133-144)	147(134-167)	155(134-172)
	1	171(155-191)	163(145-192)	40(32-51)	37(28-47)
	2	132(122-145)	122(116-128)	25(15-38)	12(-)
8	1	146(136-153)	132(128-139)	57(54-60)	51(45-57)
	2	143(137-147)	143(137-148)	41(36-48)	34(21-47)
	3	131(116-148)	113(97-142)	17(9-22)	10(3-15)

[a]Mean activities represent the average of three pooled samples from a total of 12 animals.

Table 5. (Continued)

Age (weeks)	Total weeks of exposure to date	Group I H_2O control	Group II NaCl control	Group III 125 mg/kg Pb	Group IV 250 mg/kg Pb
Mean for week					
5		165	152	151	154
6		127	127	107	105
7		154	141	71	68
8		140	129	38	32

Table 6. Body weights for control and lead exposed hamsters

Age (weeks)	Total weeks of exposure to date	Body weights[a] (grams) mean (range) Group I H_2O control	Group II NaCl control	Group III 125 mg/kg Pb	Group IV 250 mg/kg Pb	Overall[bc] week mean
5	0	63.5(50.6-72.0)	61.0(56.0-67.4)	61.0(56.3-66.5)	61.2(51.3-70.3)	
	0	61.2(57.0-67.5)	60.9(53.1-71.1)	60.7(52.2-71.7)	64.4(56.8-71.8)	61.9
	0	61.5(56.3-70.5)	61.9(55.5-68.4)	61.5(54.7-68.3)	63.6(52.6-70.6)	
6	0	82.0(67.6-93.8)	81.8(72.0-90.3)	80.1(70.4-88.9)	80.7(70.7-91.4)	
	0	77.2(70.7-86.6)	78.6(68.0-94.3)	76.6(65.2-86.1)	80.5(73.1-88.6)	77.6
	1	73.1(66.9-85.7)	75.7(64.1-88.4)	73.1(64.3-79.1)	72.3(57.8-83.5)	
7	0	89.9(74.5-102.9)	87.6(79.1-98.0)	87.4(78.1-99.3)	88.4(76.2-101.4)	
	1	87.2(78.8-98.6)	89.7(80.3-109.3)	87.3(72.1-98.3)	90.2(80.3-101.2)	86.8
	2	83.5(74.8-99.4)	85.2(69.7-100.8)	81.8(73.8-88.5)	81.8(65.5-94.3)	
8	1	98.5(82.5-115.5)	98.3(84.1-116.2)	95.4(85.4-107.1)	95.8(79.6-114.3)	
	2	92.8(84.6-105.3)	95.1(81.3-112.6)	93.6(78.7-107.4)	93.6(76.2-110.3)	93.5
	3	89.9(81.1-108.3)	92.6(76.0-108.9)	87.8(77.8-95.0)	88.7(73.3-100.1)	

[a]Body weights represent the average of 12 animals; body weights were taken for the 4 weeks prior to challenge with S. typhimurium. The final body weights were taken immediately before challenge.

[b]Dosing schedule was based on overall week mean body weights.

[c]Overall means represent the average of 144 animals for week means and the average of four observations on each of 12 animals for group means.

Table 6. (Continued)

Age (weeks)	Total weeks of exposure to date	Body weights (grams) mean (range) Group I H_2O control	Group II NaCl control	Group III 125 mg/kg Pb	Group IV 250 mg/kg Pb
Overall group means					
5		62.1	61.3	61.1	63.1
6		77.4	78.7	76.6	77.8
7		86.9	88.2	85.5	86.8
8		93.7	95.3	92.3	92.7

Figure 7. Body weights for control and lead exposed hamsters. TEX = total length of treatment exposure. Grouped by length of exposure. Body weights were taken for the 4 weeks prior to challenge with S. typhimurium. The final body weights were taken immediately before challenge.

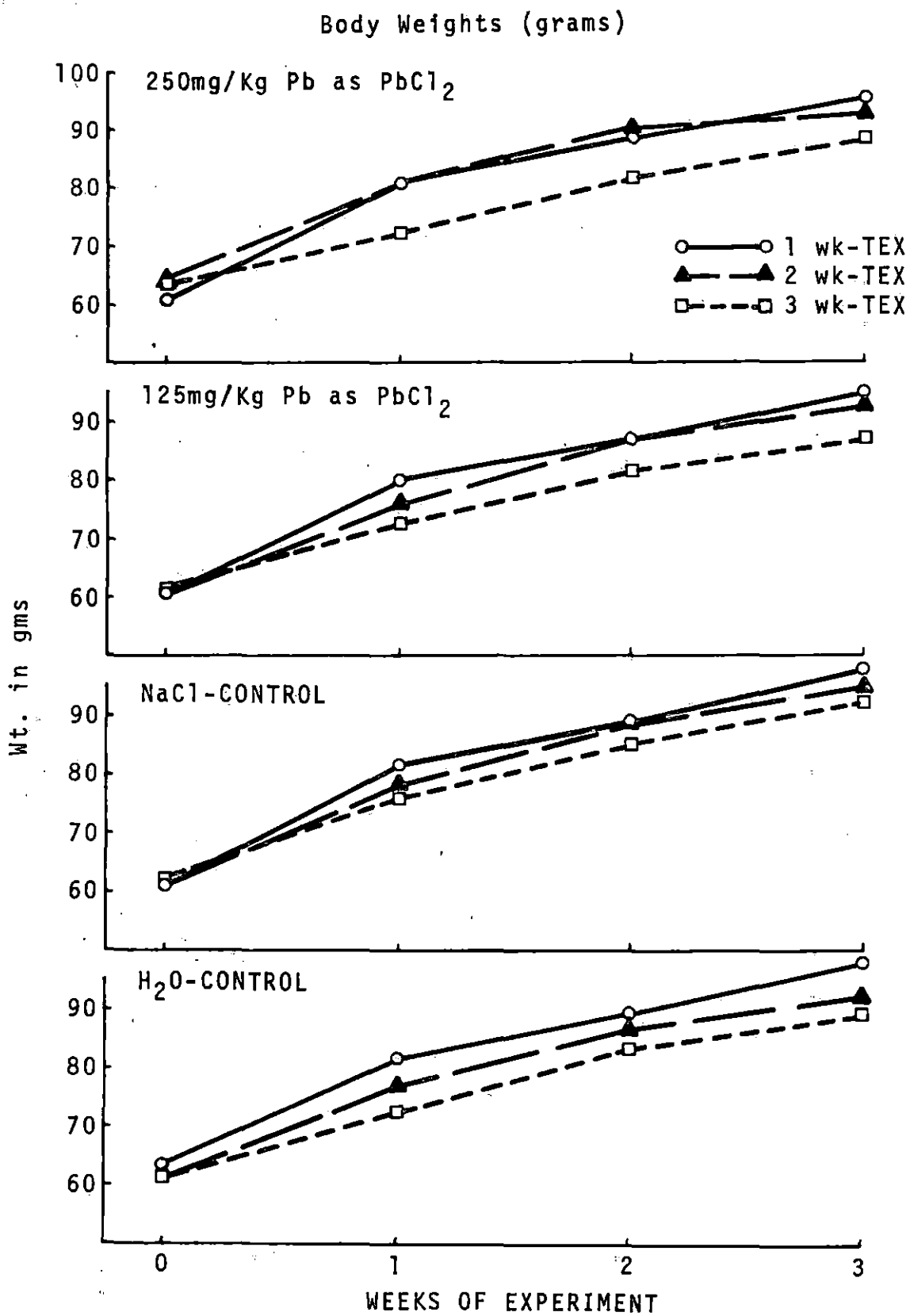
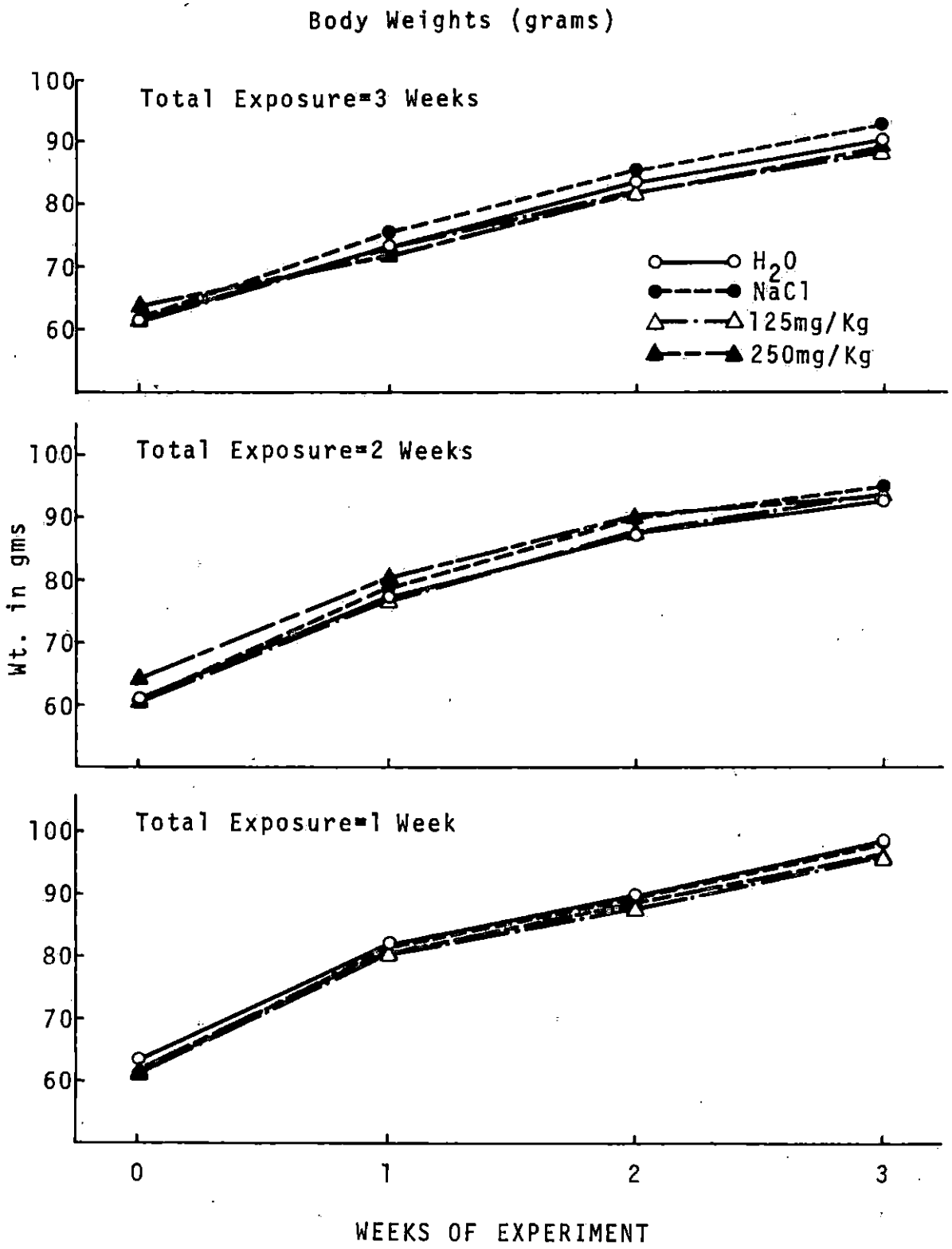

Body Weights (grams)
250mg/Kg Pb as PbCl2
125mg/Kg Pb as PbCl2
NaCl-CONTROL
H2O-CONTROL
1 wk-TEX
2 wk-TEX
3 wk-TEX
Wt. in gms
100
90
80
70
60
0
1
2
3
WEEKS OF EXPERIMENT

Figure 8. Body weights for control and lead exposed hamsters grouped by treatment. Body weights were taken for the 4 weeks prior to challenge with S. typhimurium. The final body weights were taken immediately before challenge.


Body Weights (grams)
Total Exposure=3 Weeks
Total Exposure=2 Weeks
Total Exposure=1 Week
Wt. in gms
100
90
80
70
60
H2O
NaCl
125mg/Kg
250mg/Kg
0
1
2
3
WEEKS OF EXPERIMENT

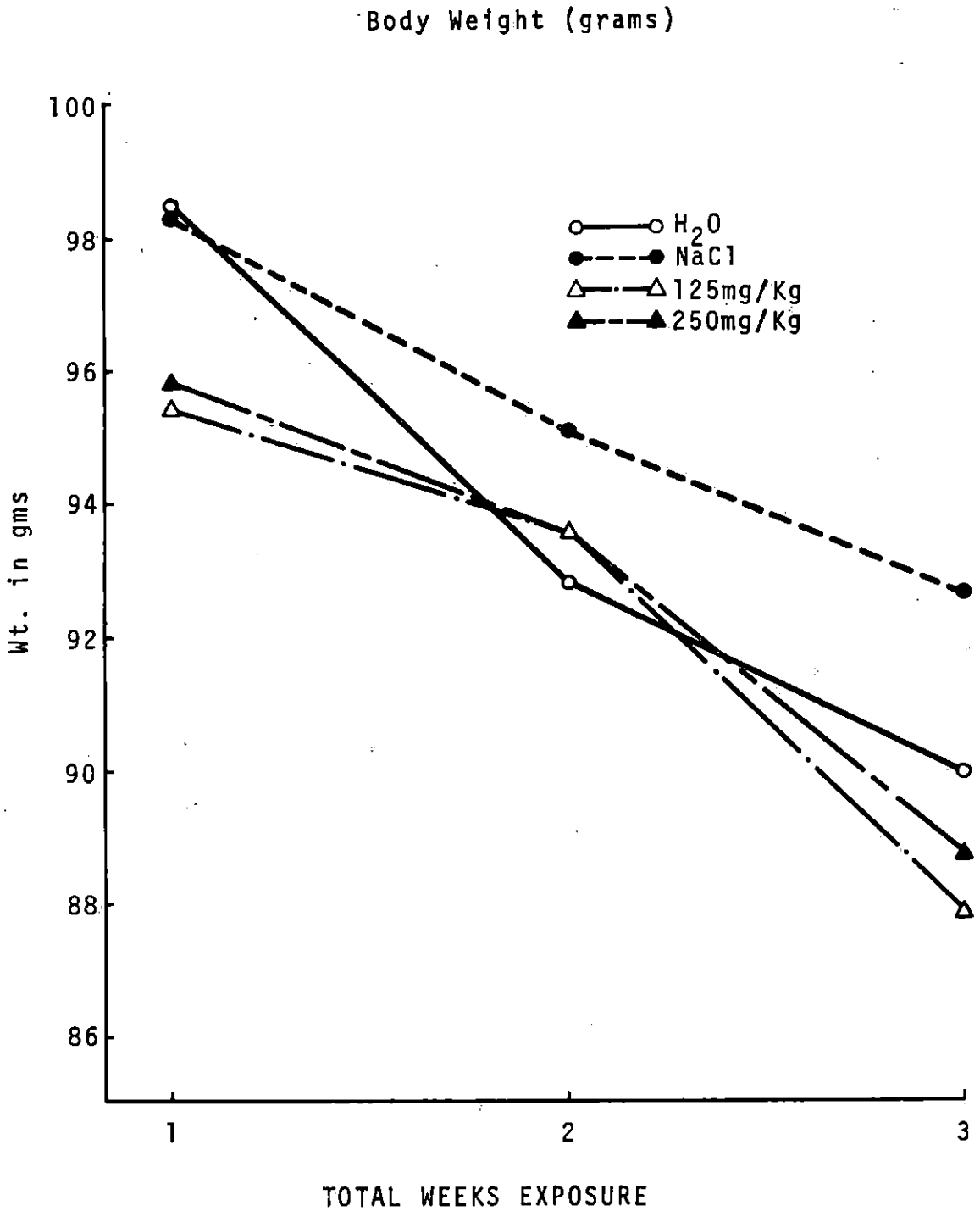


Figure 9. Final body weights for control and lead exposed hamsters as a function of total length of exposure. The final body weights were taken immediately before S. typhimurium challenge.

groups and the differences were significant at varying probability levels (Table 6 and 7).

After 1 total week of exposure the control groups tended to weigh more than the lead treated groups. After 2 weeks of total exposure these differences tended to disappear, largely due to the contribution of the H_2O control (Figure 9). At 3 weeks of total exposure the differences again appeared between the control and lead treated groups. These differences are small in terms of actual weights but the differences do appear to be real.

There was no significant decrease in the rate of gains among the lead exposed animals when compared to the control animals but all groups showed a parallel decreased rate of gains the longer they were treated (Figure 9, 10 and 11) (Table 6).

Red Blood Cell Count

There were no significant differences in RBC counts among the lead exposed and control animals (Table 8). There was a tendency in all groups toward a higher RBC count as the length of treatment exposure increased but all of the treatment groups tended to cluster and increased in parallel.

Hemoglobin Concentration

There were statistically significant differences in hemoglobin concentration between the two control groups ($P<0.01$) (Table 8). The average of the two controls was only marginally higher than the average of the two lead treatments (Table 9). This is reflected by the level of statistical

Table 7. Tests of significance for body weight

Group	Contrasts	Probability of a larger t
H_2O control (t_1)	Among all treatments	N.S.[a]
NaCl control (t_2)	Among all lengths of exposure	≈.01
125 mg/kg Pb (t_3)	TEX_1 vs TEX_2	N.S.
250 mg/kg Pb (t_4)	TEX_1 vs TEX_3	S[b]-P<.01
Total exposure of 1 week (TEX_1)	TEX_2 vs TEX_3	S-P=.05
Total exposure of 2 weeks (TEX_2)	For final body weights (Figure 9).	
	t_1*TEX_1 vs t_1*TEX_2	S-P<.01
Total exposure of 3 weeks (TEX_3)	t_1*TEX_1 vs t_1*TEX_3	N.S.
	t_1*TEX_2 vs t_1*TEX_3	S-P<.01
	t_2*TEX_1 vs t_2*TEX_2	S-P=.05
	t_2*TEX_1 vs t_2*TEX_3	S-P<.01
	t_2*TEX_2 vs t_2*TEX_3	N.S.
	t_3*TEX_1 vs t_3*TEX_2	N.S.
	t_3*TEX_1 vs t_3*TEX_2	S-P<.01
	t_3*TEX_2 vs t_3*TEX_3	S-P<.01
	t_4*TEX_1 vs t_4*TEX_2	N.S.
	t_4*TEX_1 vs t_4*TEX_3	S-P<.01
	t_4*TEX_2 vs t_4*TEX_3	S-P<.01

[a]No significant difference.

[b]Significant.

Table 7. (Continued)

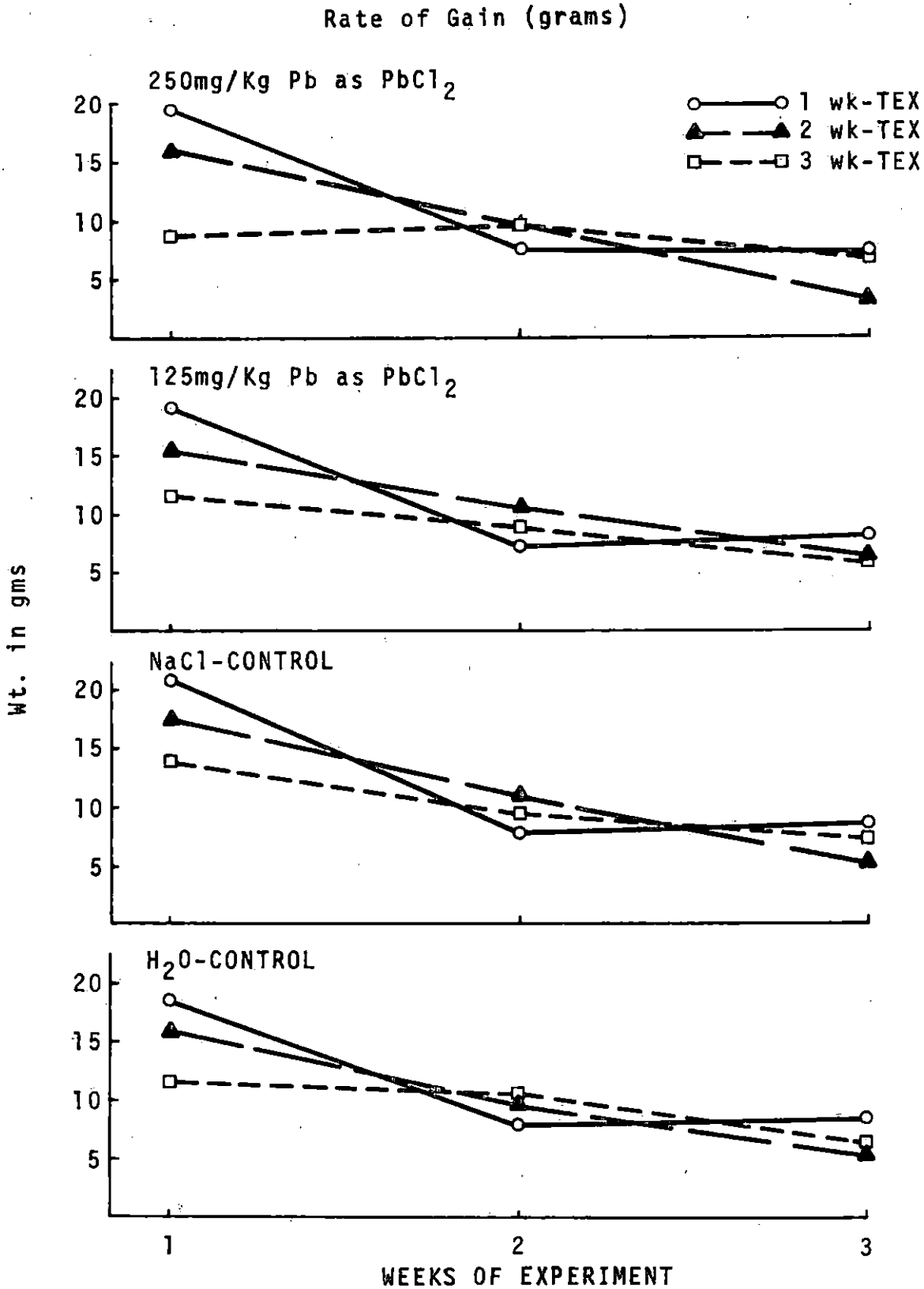
Group	Contrasts	Probability of a larger t
H_2O control (t_1)	For final body weights (Figure 9)	
NaCl control (t_2)	$\frac{t_1*TEX_1+t_2*TEX_1}{2}$ vs $\frac{t_3*TEX_1+t_4*TEX_1}{2}$	S-P<.01
125 mg/kg Pb (t_3)	$\frac{t_1*TEX_2+t_2*TEX_2}{2}$ vs $\frac{t_3*TEX_2+t_4*TEX_2}{2}$	N.S.
250 mg/kg Pb (t_4)	$\frac{t_1*TEX_3+t_2*TEX_3}{2}$ vs $\frac{t_3*TEX_3+t_4*TEX_3}{2}$	S-P<.01

significance (P=.02) and the means ($\overline{X}$-control=15.1 g/100 ml, $\overline{X}$-lead exposed=14.8 g/100 ml). The NaCl control was significantly higher than the average of the other three treatments (P<0.01). This difference remained throughout all three lengths of exposure. The tendency was for the hemoglobin concentrations to increase as the length of exposure increased within the two lead treatment and the control groups (Table 9).

A regression analysis was conducted on the data and very little of the increase in hemoglobin concentration could be explained by an increase in RBC count. In the H_2O control about 5% (R^2=.05)[1] of the observed increase in hemoglobin concentration could be explained by an increase in

[1] R^2 = coefficient of determination; the square of the correlation coefficient. In regression analysis it represents the proportion of a total sums of squares that is attributed to another source of variation. Steel, R. G. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York. p. 187.

Figure 10. Weekly rate of gains for control and lead exposed hamsters. Grouped by length of exposure. Body weights were taken for the 4 weeks prior to challenge with S. typhimurium.


Rate of Gain (grams)
250mg/Kg Pb as PbCl2
125mg/Kg Pb as PbCl2
NaCl-CONTROL
H2O-CONTROL
1 wk-TEX
2 wk-TEX
3 wk-TEX
Wt. in gms
20
15
10
5
1
2
3
WEEKS OF EXPERIMENT

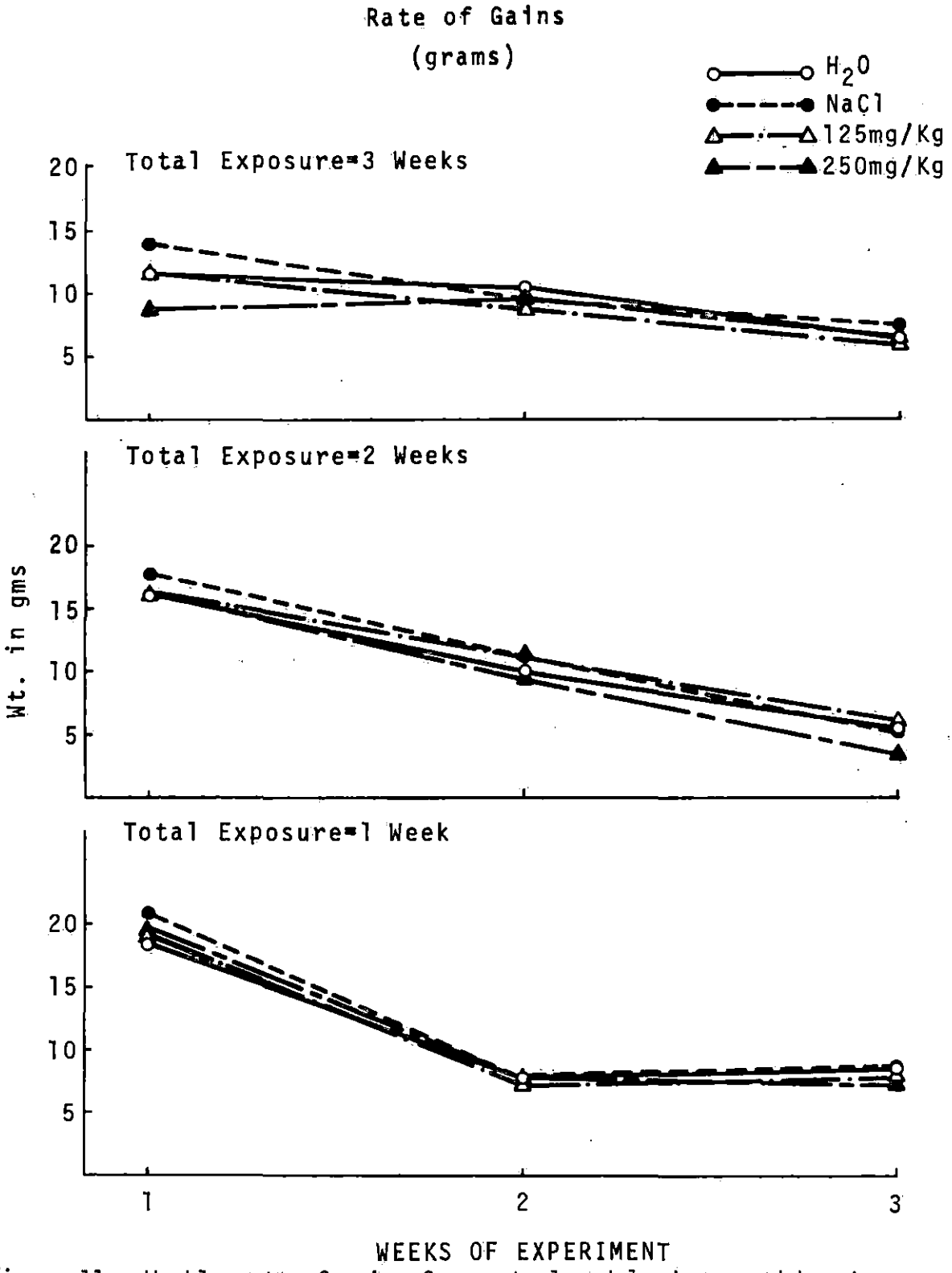


Figure 11. Weekly rate of gains for control and lead exposed hamsters grouped by treatment. Body weights were taken for the 4 weeks prior to challenge with S. typhimurium.

Table 8. Characteristics of hamster red cells[a]

		H_2O control	NaCl control	125 mg/kg Pb	250 mg/kg Pb
Hematocrit (%)	TEX[b] = 1;	41(39-45)	42(39-44)	41(39-43)	41(39-43)
	TEX = 2;	41(39-43)	42(41-43)	42(40-43)	40(38-42)
	TEX = 3;	42(38-43)	43(39-45)	42(38-43)	42(40-43)
Red cell count (10^6/ul)	TEX = 1;	5.92(5.73-6.10)	5.90(5.50-6.17)	5.87(5.63-6.13)	5.73(5.47-6.20)
	TEX = 2;	5.61(4.40-6.57)	5.63(4.43-6.70)	5.78(4.20-6.73)	5.80(4.83-6.63)
	TEX = 3;	6.09(5.33-6.87)	5.83(5.40-6.17)	6.25(5.40-7.07)	6.22(5.40-7.43)
Hemoglobin (g/100 ml)	TEX = 1;	14.9(14.3-15.9)	15.2(14.8-15.6)	14.6(14.0-15.5)	14.3(13.6-15.1)
	TEX = 2;	14.8(14.1-15.5)	15.3(14.3-16.0)	15.1(14.3-15.9)	14.6(13.5-15.3)
	TEX = 3;	15.1(13.7-15.9)	15.4(14.1-15.9)	15.3(14.1-15.9)	15.0(14.1-15.5)
Mean corpuscular hemoglobin (picograms)	TEX = 1;	26(24-28)	26(24-28)	25(24-26)	25(22-27)
	TEX = 2;	27(21-33)	28(21-35)	27(21-35)	26(20-32)
	TEX = 3;	25(20-29)	27(23-29)	25(20-29)	25(19-29)
Mean corpuscular volume (u^3)	TEX = 1;	70(64-78)	71(63-80)	71(64-73)	71(63-75)
	TEX = 2;	75(59-98)	78(61-97)	75(59-98)	70(57-87)
	TEX = 3;	69(55-81)	74(63-80)	67(54-80)	69(54-78)

[a]Mean (range); week mean represents the average across all weeks; each mean is the average of 12 pooled observations from three cages over a period of 4 weeks. Each case contained four animals and yielded one pooled sample per week.

[b]TEX = total weeks of exposure to treatment.

Table 9. Tests of significance for hemoglobin data

Group	Contrasts	Probability of a larger t
H_2O control (t_1)	t_1 vs t_2	P<.01
	t_1 vs t_3	N.S.[a]
NaCl control (t_2)	t_1 vs t_4	P=.01
	t_2 vs t_3	P=.01
125 mg/kg Pb (t_3)	t_2 vs t_4	P<.01
	t_3 vs t_4	P=.01
250 mg/kg Pb (t_4)	$\frac{t_1+t_2}{2}$ vs $\frac{t_3+t_4}{2}$	P=.02
	t_2 vs $\frac{t_1+t_3+t_4}{3}$	P<.01
Total exposure of 1 week (TEX_1)	TEX_1 vs TEX_2	P=.05
Total exposure of 2 weeks (TEX_2)	TEX_1 vs TEX_3	P<.01
Total exposure of 3 weeks (TEX_3)	TEX_2 vs TEX_3	P=.02
Interactions	$\frac{t_1*TEX_3+t_2*TEX_3}{2}$ vs $\frac{t_3*TEX_3+t_4*TEX_3}{2}$	N.S.
	t_3*TEX_3 vs t_4*TEX_3	N.S.

[a]No significant difference.

RBC count. Likewise only 9% (R^2=.09) of the increase in hemoglobin concentration could be explained by an increase in RBC count in the NaCl control group. In the lead treated groups less than 1% (R^2=.001) could be explained in the 125 mg/kg group and about 7% (R^2=.07) of the hemoglobin concentration increase could be explained by a parallel increase in RBC in the 250 mg/kg group.

There were no statistically significant differences among the treatment means for mean corpuscular hemoglobin and mean corpuscular volume throughout the experiment (Table 8).

The hemoglobin concentrations did not correlate very highly with blood lead levels: H_2O control, 0.27; NaCl control, 0.05; 125 mg/kg, 0.80; and 250 mg/kg, 0.40.

Hematocrit

There were no statistically significant differences in hematocrit among the experimental groups (Tables 8 and 10). The hematocrit of the NaCl control tended to be slightly higher than the other three treatments.

There was little correlation between the hematocrit and in RBC count: H_2O control, -0.41; NaCl control, -0.23; 125 mg/kg Pb, -0.24; and 250 mg/kg Pb, -0.19.

White Cell Count

There were no significant differences among the treatment groups for the following parameters: total white blood cell count, % neutrophiles, % bands, % lymphocytes, % monocytes, and % eosinophiles (Table 11).

Serum Protein

There were no significant differences among the treatment groups or lengths of exposure in total serum protein values (g/100 ml) and the percentages of each fraction (Tables 12 and 13).

Table 10. Tests of significance for hematocrit data

Group	Contrasts	Probability of a larger t
H_2O control (t_1)	t_1 vs t_2	P=.01
	t_1 vs t_3	N.S.[a]
NaCl control (t_2)	t_1 vs t_4	N.S.
	t_2 vs t_3	P=.05
125 mg/kg Pb (t_3)	t_2 vs t_4	P<.01
	t_3 vs t_4	N.S.
250 mg/kg Pb (t_4)	$\frac{t_1+t_2}{2}$ vs $\frac{t_3+t_4}{2}$	N.S.
	t_2 vs $\frac{t_1+t_3+t_4}{3}$	P=.02, almost S.[b] at (.01)
Total exposure of 1 week (TEX_1)	TEX_1 vs TEX_2	N.S.
Total exposure of 2 weeks (TEX_2)	TEX_1 vs TEX_3	P=.02
Total exposure of 3 weeks (TEX_3)	TEX_2 vs TEX_3	P=.05
Interactions	$\frac{t_1*TEX_3+t_2*TEX_3}{2}$ vs $\frac{t_3*TEX_3+t_4*TEX_3}{2}$	N.S.
	t_3*TEX_3 vs t_4*TEX_3	N.S.

[a]No significant difference.

[b]Significant.

Table 11. Differential count for control and lead exposed hamsters, mean (range)

		H_2O control	NaCl control	125 mg/kg Pb	250 mg/kg Pb
White blood cell count	TEX_1	6600(6000-7200)	6600(6400-7100)	7200(6500-7700)	8100(6800-10,300)
	TEX_2	5700(5100-6200)	7300(5600-10,300)	6100(6000-6300)	6200(5400-6800)
	TEX_3	6900(6600-7200)	6500(6200-6900)	6900(6200-7400)	7300(6700-7900)
% neutrophile	TEX_1	26(22-30)	25(23-27)	29(27-30)	29(26-31)
	TEX_2	28(26-30)	25(23-26)	21(19-23)	23(21-26)
	TEX_3	24(17-30)	25(22-28)	27(18-36)	26(25-29)
% neutrophile bands	TEX_1	4(3-6)	6(5-7)	5(4-6)	3(2-4)
	TEX_2	3(2-4)	4(3-5)	6(3-9)	3(2-4)
	TEX_3	3(1-6)	4(2-5)	3(2-5)	3(1-4)
% lymphocyte	TEX_1	69(67-72)	69(67-72)	65(64-68)	68(66-72)
	TEX_2	69(64-72)	70(69-73)	73(67-76)	73(72-75)
	TEX_3	73(68-81)	71(70-73)	70(62-77)	71(68-74)
% monocyte	TEX_1	0(0-1)	1(1-2)	0(0-1)	0(0-2)
	TEX_2	1(0-3)	1(1-3)	1(1-2)	1(0-2)
	TEX_3	1(0-2)	1(0-3)	0(0-1)	0(0-1)
% eosinophile	TEX_1	1(0-2)	0(0-1)	0(0-1)	0(0-1)
	TEX_2	0(0-1)	1(0-1)	0(0)	0(0-1)
	TEX_3	0(0-1)	0(0)	0(0-1)	0(0)

Table 12. Mean total serum proteins before and after lead exposure (g/100 ml)[a]

Age (weeks)	Total weeks of exposure to date	Group I H_2O control	Group II NaCl control	Group III 125 mg/kg Pb	Group IV 250 mg/kg Pb	Before vs after week means
5	0	-	-	-	-	
	0	-	-	-	-	
	0	6.6	6.8	6.8	6.5	
6	0	-	-	-	-	
	0	6.1	6.3	6.4	6.3	
	1	-	-	-	-	
7	0	6.6	6.7	6.9	7.0	
	1	-	-	-	-	6.6
	2	-	-	-	-	n=36
8	1	6.1	6.0	6.1	6.1	
	2	6.8	6.9	7.0	7.1	6.4
	3	6.5	6.0	6.2	6.1	n=36
Overall group means[b]		6.5	6.5	6.6	6.5	
		n=36	n-36	n=36	n=36	

[a]Serum sample means represent the average from three pooled samples from a total of 12 animals for each value above. Serum samples were taken only twice during the experiment; once 24 hrs before exposure began and 24 hrs after the final lead dose.

[b]Overall week and group means represent the average of 36 pooled observations from a total of 144 animals.

Table 13. Mean characteristics of hamster serum before and after lead exposure[a]

		H_2O control		NaCl control		125 mg/kg Pb		250 mg/kg Pb	
		Before	After	Before	After	Before	After	Before	After
Total protein (g/100ml)	TEX[b] = 1	6.6	6.1	6.7	6.0	6.1	6.1	7.0	6.1
	TEX = 2	6.1	6.8	6.3	6.9	6.4	7.0	6.3	7.1
	TEX = 3	6.6	6.5	6.8	6.0	6.8	6.2	6.5	6.1
Albumin (%)	TEX = 1	43.07	49.70	43.93	49.27	42.07	52.03	41.47	49.57
	TEX = 2	40.97	46.83	47.43	44.10	41.73	48.83	43.40	44.93
	TEX = 3	47.87	53.00	53.13	42.67	47.87	49.27	44.87	45.33
$Alpha_1$ globulin (%)	TEX = 1	18.33	16.03	18.63	13.27	17.43	13.90	17.97	14.77
	TEX = 2	20.10	14.23	16.13	13.63	16.50	14.13	17.20	12.80
	TEX = 3	15.70	14.20	16.00	16.27	17.50	14.57	17.60	16.37
$Alpha_2$ globulin (%)	TEX = 1	11.23	10.70	11.20	13.27	9.63	11.97	9.93	11.90
	TEX = 2	8.33	13.50	8.70	17.10	9.80	11.43	9.47	14.40
	TEX = 3	12.27	9.10	9.67	13.10	10.20	10.53	11.33	10.20
Beta globulin (%)	TEX = 1	14.90	15.50	14.03	16.33	20.70	14.37	16.30	14.53
	TEX = 2	19.50	15.73	19.27	15.83	20.47	16.50	19.47	17.77
	TEX = 3	15.20	10.80	13.53	16.73	15.07	15.10	15.73	18.00
Gamma globulin (%)	TEX = 1	12.37	8.07	12.17	7.87	10.20	7.73	13.37	9.23
	TEX = 2	11.10	9.70	8.47	9.33	11.50	9.10	10.47	10.10
	TEX = 3	9.00	7.50	7.67	11.23	9.37	10.53	10.47	10.10

[a]Each mean represents the average of three pooled samples of hamster serum from a total of 12 animals. Serum samples were taken 24 hours before initial lead exposure and 24 hours after the last lead exposure (immediately before S. typhimurium challenge).

[b]TEX = total weeks of exposure to lead.

Mortality

The mean percent mortality for the lead exposed and control groups were as follows: H_2O control, 69.4%; NaCl control, 57.3%; 125 mg/kg Pb, 63.9%; and 250 mg/kg Pb, 63.9%. These means were not significantly different (Figure 12). The mean percent mortalities in all groups combined for total length of treatment exposure were 59.7%, 62.5% and 68.8% for the 1, 2 and 3 weeks, respectively. These means were not significantly different (Table 14).

The NaCl control showed little variance in percent mortality as the length of exposure increased (Figure 13). There was a tendency for percent mortality to increase in both lead treated groups as the length of treatment exposure increased. The increase in percent mortality in the 125 mg/kg Pb treatment was not significant and the correlation between the percent mortality and the total length of treatment exposure was 0.14. In the 250 mg/kg Pb exposure the increase in percent mortality also was not significant and the correlation between the percent mortality and total length of exposure in this group was 0.40. Thus, there was a very small tendency for the percent mortality to increase as the total length of exposure increased.

Times of Death

The mean times of death for the four treatments were found to be 46.2 hours, 41.8 hours, 49.1 hours and 46.6 hours for the H_2O control, NaCl control, 125 mg/kg Pb and 250 mg/kg Pb treatments, respectively. These means were not significantly different. The mean times of death for

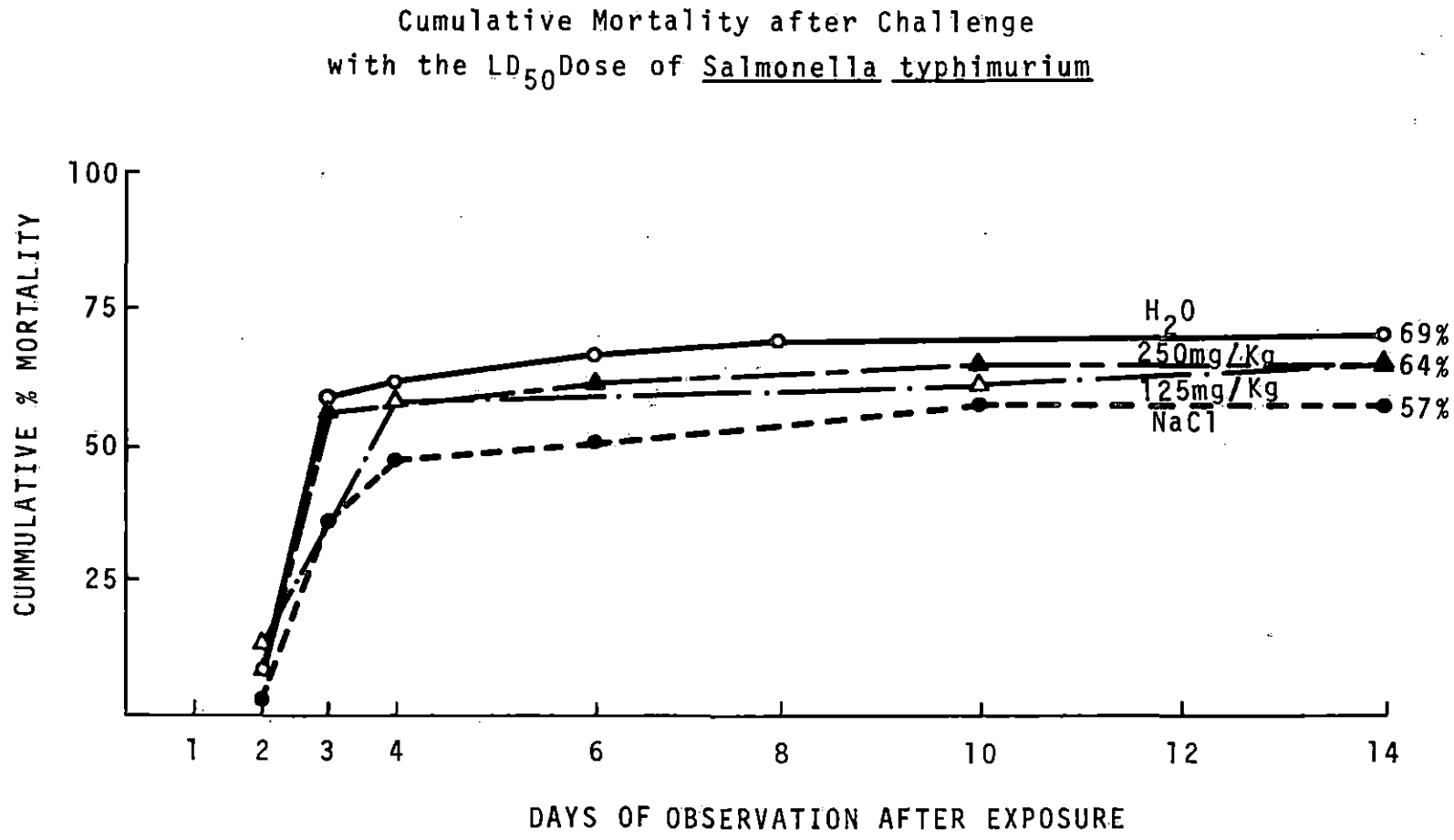
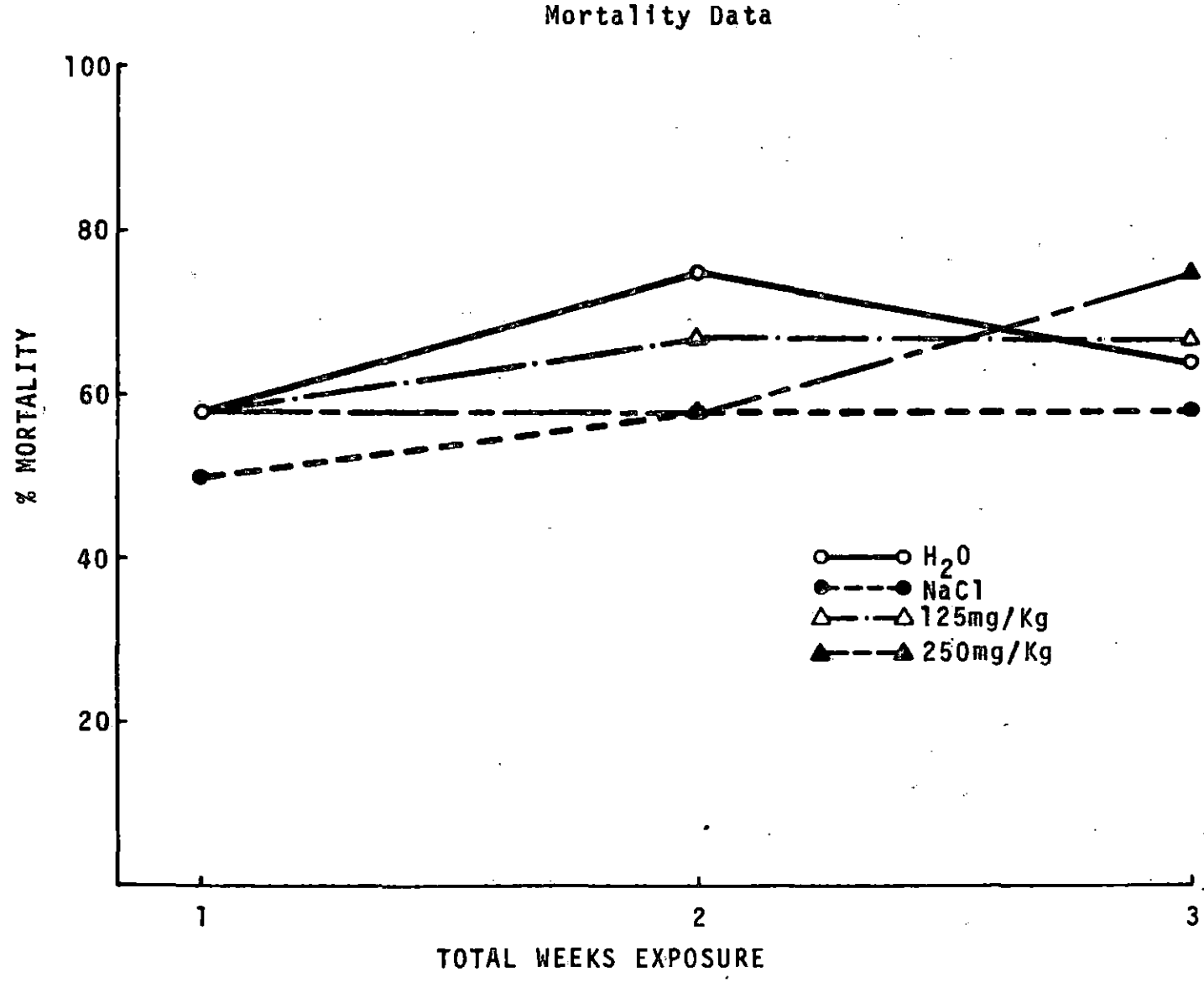


Figure 12. Cumulative mortality data for control and lead exposed hamsters subsequent to S. typhimurium challenge.

Table 14. Mortality data for control and lead exposed hamsters (mean percent mortality)

Age at initial exposure to treatment (weeks)	Total length of treatment (weeks)	Group I	Group II	Group III	Group IV	Total length of exposure
5	3	75.0	58.3	66.7	75.0	68.8
6	2	75.0	50.0	66.7	58.3	62.5
7	1	58.3	63.7	58.3	58.3	59.7
Group means		69.4	57.3	63.9	63.9	

Figure 13. Mortality data for control and lead exposed hamsters by total length of treatment exposure subsequent to S. typhimurium challenge.


Mortality Data
100
80
60
40
20
% MORTALITY
1
2
3
TOTAL WEEKS EXPOSURE
H2O
NaCl
125mg/Kg
250mg/Kg

the three lengths of treatment exposure were found to be 50.8 hours, 41.6 hours and 45.4 hours. These means were not significantly different. The animals in the four treatment groups died at about the same rate (Figure 12, Table 15).

Hepatic Tissue Lead

The mean hepatic lead levels for the control and lead exposed groups were: H_2O control, 1.46 µg/gm; NaCl control, 1.38 µg/gm; 125 mg/kg Pb, 2.74 µg/gm; and 250 mg/kg Pb, 4.75 µg/gm. The levels in the lead exposed animals were significantly higher than the controls.

The hepatic tissue lead levels for the three length of treatment exposure were: 1 week, 1.95 µg/gm; 2 weeks, 2.62 µg/gm; and 3 weeks, 3.18 µg/gm. The tissue lead level of 3 weeks of exposure was significantly elevated while at 2 weeks it was not significantly higher than at 1 week.

After 1 week of lead exposure the average hepatic tissue lead for the two controls was significantly less than the average of the two lead treatments but the 250 mg/kg Pb treatment was not significantly higher than the 125 mg/kg Pb treatment group. The H_2O control group had hepatic lead levels of 0.92 µg/gm, 1.69 µg/gm and 1.78 µg/gm for the total lengths of treatment exposure of 1, 2 and 3 weeks, respectively. The NaCl control group had hepatic tissue lead levels of 1.36 µg/gm, 1.48 µg/gm and 1.28 µg/gm for 1, 2 and 3 weeks of treatment exposure, respectively. The increase in the hepatic tissue lead levels for the H_2O control and for the NaCl control were not significant.

Table 15. Mean times of death for control and lead exposed hamsters (hours)

Age at initial exposure to treatment (weeks)	Total length of treatment (weeks)	Group I	Group II	Group III	Group IV	Total length of exposure
5	3	42.8	54.1	39.8	44.8	45.4
6	2	48.5	33.0	36.6	48.4	41.6
7	1	47.3	38.3	70.9	46.8	50.8
Group means		46.2	41.8	49.1	46.6	

The hepatic tissue lead levels for the 125 mg/kg Pb group were 2.33 µg/gm, 2.66 µg/gm and 3.23 µg/gm for the total exposure lengths of 1, 2 and 3 weeks, respectively. The increases in hepatic tissue lead for the 125 mg/kg Pb group for the three lengths of treatment exposure were not significantly different. These increases were significantly higher than the control hepatic tissue levels but the increase over time was not significant. The mean hepatic tissue lead levels in the 250 mg/kg group were 3.20 µg/gm, 4.63 µg/gm and 6.41 µg/gm for 1, 2 and 3 weeks of treatment exposure, respectively. The hepatic tissue lead level at 1 week of total exposure in the 250 mg/kg Pb group was not significantly higher than the 125 mg/kg Pb group but it was significantly higher than the control groups ($P<.01$). At 2 weeks of total exposure the 250 mg/kg Pb group showed a significantly higher hepatic tissue level than the 125 mg/kg Pb group ($P<.01$). At 3 weeks of total exposure the 250 mg/kg Pb group showed hepatic tissue lead levels that were significantly higher than the levels found in the 125 mg/kg Pb group ($P<.001$) (Table 16, Figure 14). The correlation between blood lead and hepatic tissue lead level was 0.99.

Renal Tissue Lead Levels

The mean H_2O control renal tissue lead levels were 1.42 µg/gm, 1.55 µg/gm and 1.81 µg/gm for 1, 2 and 3 weeks of treatment exposure (Table 17, Figure 15). The increase in renal tissue lead in the H_2O control was not significant. The mean renal tissue lead levels for the NaCl control were 1.63 µg/gm, 2.42 µg/gm and 1.63 µg/gm for 1, 2 and 3 weeks of treatment exposure, respectively. The increase in the renal tissue lead burden for

Table 16. Tests of significance for hepatic tissue lead levels

Group	Contrasts	Probability of a larger t
H_2O control (t_1)	t_1 vs t_2	N.S.[a]
NaCl control (t_2)	t_1 vs t_3	P<.01
125 mg/kg Pb (t_3)	t_1 vs t_4	P<.001
250 mg/kg Pb (t_4)	t_2 vs t_3	P<.01
	t_2 vs t_4	P<.001
	t_3 vs t_4	P<.001
	$\frac{t_1+t_2}{2}$ vs $\frac{t_3+t_4}{2}$	P<.001
Total exposure of 1 week (TEX_1)	TEX_1 vs TEX_2	P<.05
Total exposure of 2 weeks (TEX_2)	TEX_1 vs TEX_3	P<.001
Total exposure of 3 weeks (TEX_3)	TEX_2 vs TEX_3	N.S.
	TEX_1 vs $\frac{TEX_2+TEX_3}{2}$	P<.01
Interactions	$\frac{t_1*TEX_1+t_2*TEX_1}{2}$ vs $\frac{t_3*TEX_1+t_4*TEX_1}{2}$	P<.01

[a]No significant difference.

the 2 weeks of total exposure group was not significant. The mean renal tissue lead levels for the 125 mg/kg Pb treatment group were 2.18 µg/gm, 3.63 µg/gm and 4.92 µg/gm for 1, 2 and 3 weeks of treatment exposure, respectively. The increase in the renal tissue lead was not significantly higher at 2 weeks exposure than at 1 week of exposure (Table 17). The

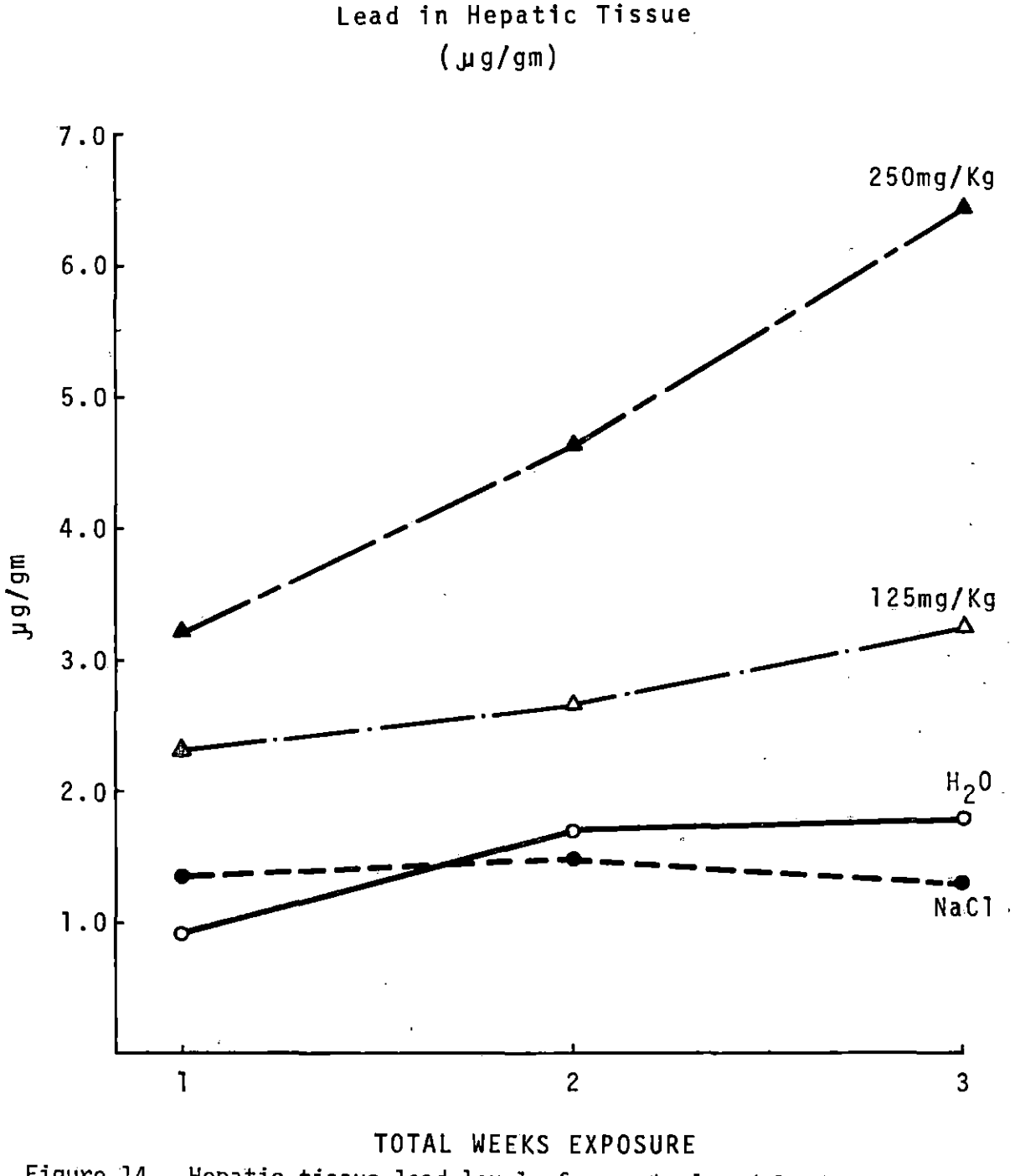


Figure 14. Hepatic tissue lead levels for control and lead exposed hamsters by total length of treatment exposure; tissues taken after S. typhimurium challenge - observation period.

Table 17. Tests of significance for renal tissue lead levels

Group	Contrasts	Probability of a larger t
H_2O control (t_1)	t_1 vs t_2	N.S.[a]
NaCl control (t_2)	t_1 vs t_3	P<.01
125 mg/kg Pb (t_3)	t_1 vs t_4	P<.001
250 mg/kg Pb (t_4)	t_2 vs t_3	P<.05
	t_2 vs t_4	P<.01
	t_3 vs t_4	N.S.
	$\frac{t_1+t_2}{2}$ vs $\frac{t_3+t_4}{2}$	P<.001
Total exposure of 1 week (TEX_1)	TEX_1 vs TEX_2	N.S.
Total exposure of 2 weeks (TEX_2)	TEX_1 vs TEX_3	P<.01
Total exposure of 3 weeks (TEX_3)	TEX_2 vs TEX_3	N.S.
Interactions	$\frac{t_1*TEX_3+t_2*TEX_3}{2}$ vs $\frac{t_3*TEX_3+t_4*TEX_3}{2}$	P<.001

[a]No significant difference.

increase in the renal tissue lead level was not significant between the 2 and 3 weeks of exposure but 3 weeks of exposure was significantly higher than 1 week of exposure ($P<0.05$). The mean renal tissue lead levels for the 250 mg/kg Pb treatment group were 2.94 µg/gm, 3.78 µg/gm and 6.41 µg/gm for 1, 2 and 3 weeks of treatment exposure, respectively. The means for 1 and 2 weeks of exposure were not significantly different. The 3 weeks

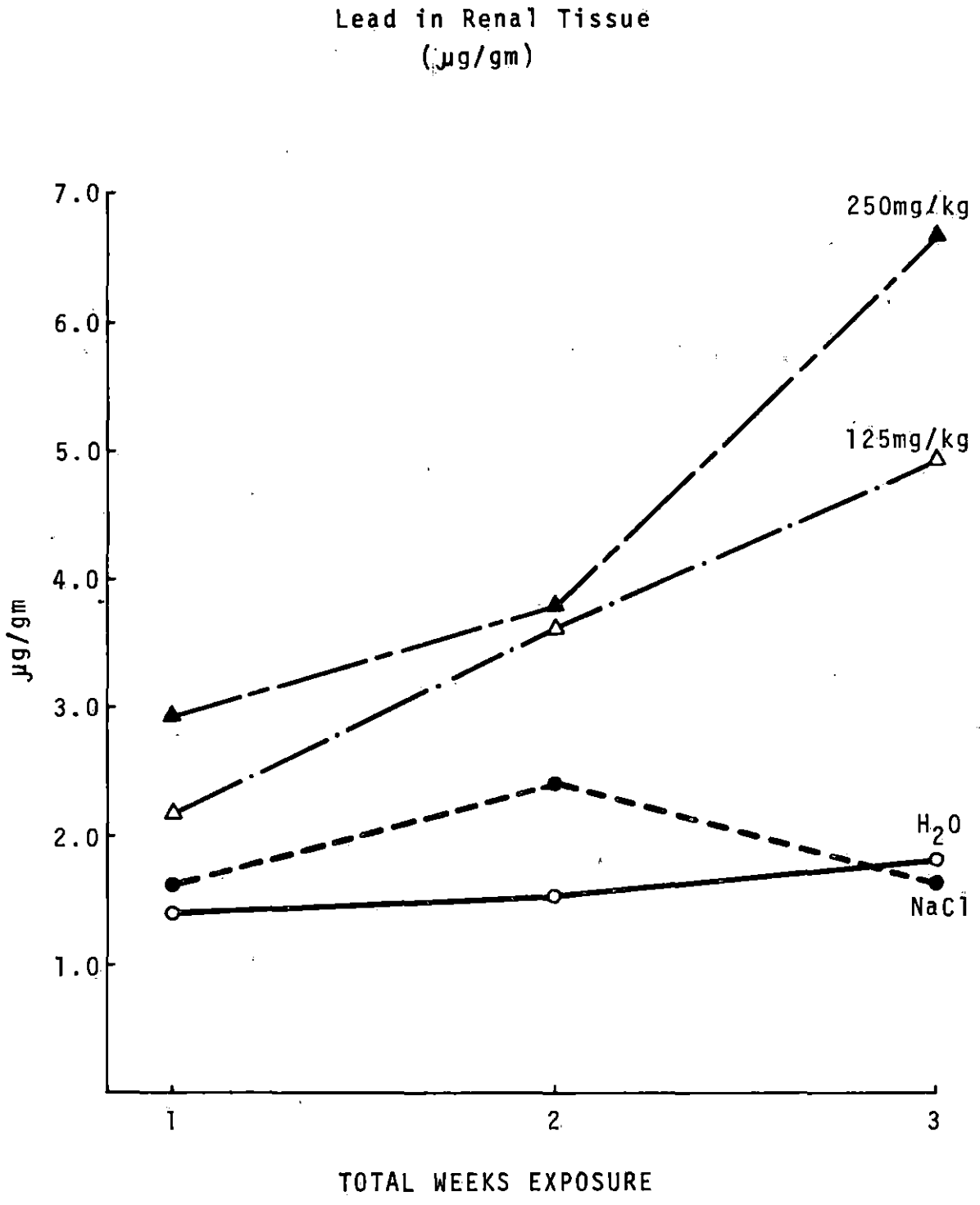


Figure 15. Renal tissue lead levels for control and lead exposed hamsters by total length of treatment exposure; tissues taken after S. typhimurium challenge - observation period.

of exposure resulted in a significantly higher renal tissue lead level than the 1 and 2 week exposure groups at the 1% and 5% levels, respectively.

The overall mean renal tissue lead levels for the four treatment groups were: H_2O control, 1.59 µg/gm; NaCl, 1.89 µg/gm; 125 mg/kg Pb, 3.58 µg/gm; and 250 mg/kg Pb, 4.46 µg/gm. The control levels were not significantly different from one another and the two lead exposed groups were not significantly different. The average renal tissue lead level in the two lead treatments was significantly higher than the average in the two control groups (Table 17).

The mean renal tissue lead levels for the three lengths of treatment exposure were 2.04 µg/gm, 2.85 µg/gm and 3.75 µg/gm for 1, 2 and 3 weeks of treatment exposure, respectively. The levels in the 3 weeks exposure group were significantly higher than the 1 week exposure group but not the 2 weeks exposure group. The levels in the 2 weeks exposure group were not significantly higher than the 1 week group. The 250 mg/kg Pb renal tissue levels were not significantly higher than the 125 mg/kg Pb treatment group.

After 1 and 2 weeks of treatment exposure the renal tissue lead levels did not differ significantly among the four treatment groups. After 3 weeks of total treatment exposure the average of the two lead treatments was significantly higher than the average of the two control group ($P<0.01$). The correlation between the renal tissue lead level and the observed blood lead level was 0.98.

DISCUSSION AND CONCLUSIONS

The paramount interest in the conduct of this study was to evaluate the effect of lead on the survival of hamsters exposed to both lead and S. typhimurium. Recent research had shown that mice exposed to 250 and 100 µg/20 gm mouse (5 mg/kg and 12.5 mg/kg, respectively) lead as lead acetate via intraperitoneal injection had a significantly higher mortality following S. typhimurium challenge than controls (Hemphill et al., 1971) and lead acetate given I.V. to young chicks enhanced the toxicity of E. coli endotoxin (Truscott, 1970). Other environmental toxicants have also been reported to reduce normal host defenses. Mallard ducks had a significantly higher mortality when exposed to polychlorinated biphenyl and duck hepatitis virus (Friend and Trainer, 1970) and carbon dust caused a reduction in the number of antibody forming cells in exposed mice (Zarkower and Morges, 1972). The literature is not replete with examples of chemically potentiated diseases. There appears to be an equal number of examples, however, where no significant effect was attributable to the toxicant being investigated. Chickens exposed to levels of lead as high as 160 mg/kg/day for 35 days failed to show reduced antibody production to Newcastle disease virus (Vengris and Maré, 1974) and hamsters, among other species, exposed to automotive exhaust for up to 23 months did not have significantly higher morbidity or mortality than controls (Hueter et al., 1966). The study reported herein demonstrated that the hamster is among the species that are more highly resistant to the effects of lead induced immunosuppression.

The whole blood lead levels in the lead exposed groups were significantly higher than the controls. At exposure levels of 125 and 250 mg/kg/day, blood levels as high as 83 and 214 µg/100 ml, respectively, were achieved. The controls exhibited a slight increase in blood lead but this was not significant. The blood lead levels had some variations with age at initial exposure. Those animals exposed to lead at 7 weeks of age to 250 mg/kg/day had the highest levels after 1 week of exposure. This may represent a higher rate of absorption or a slower rate of sequestering of lead. Other research had shown that in the rat there was a marked drop in the absorption of lead immediately after weaning (Forbes and Reina, 1972). A marked effect of dietary calcium on the absorption rate of lead has been reported (Mahaffey et al., 1973). Low calcium not only increased the blood lead levels but it also resulted in more pronounced indications of lead intoxication such as a higher rate of ALA excretion in the urine. The presence of food in the G.I. tract has been shown to decrease the rate of absorption of lead while the presence of natural chelating agents increased its absorption (Garber and Wei, 1974). The results of the present study would appear to reflect a slower rate of removal of lead since there are no indications that animals have an increased susceptibility to lead as they mature. From all indications, the rate of absorption of lead is between 5-10% of the ingested dose over a wide range of intake. The significant increases in hepatic and renal lead content were more dependent on the size of the daily dose than the age at initial exposure or length of exposure. This is consistent with other studies which have shown increased retention of lead with increasing dosage (Mahaffey et al., 1973; Goyer et al., 1970).

ALAD catylizes the formation of 2 moles of ALAD into porphobilinogen. This is the immediate precursor of the porphyrins. It has been assumed that this was the major pathway in the synthesis of hemoglobin. Based on this assumption, if the enzyme ALAD was inactivated there should be a drop in the hemoglobin level in proportion to the degree of enzyme inactivation. This study has shown that at both levels of lead exposure this enzyme was reduced in activity from an average of 155 units to less than 20 units after 3 weeks of exposure. This was accompanied by small but significant changes in the hemoglobin concentration ($P=0.01$) and the hematocrit ($P=0.03$). The lead exposed animals tended to have lower hemoglobin concentrations than the controls and the 250 mg/kg group tended to be lower than the 125 mg/kg group. There was no pattern in the hematocrit data except that the NaCl control group tended to be higher than the other three groups. Since there are no known body reserves of hemoglobin it was expected that the inactivation of the enzyme ALAD would produce a proportional reduction in hemoglobin. This did not occur. In fact, there was an increase in hemoglobin as the length of exposure increased and this increase was not due to increased hematopoiesis. Other small decreases in hemoglobin have been reported (Rogers et al., 1971) and it has been suggested that the inconsistencies in these data (larger decreases in ALAD than hemoglobin) may be a reflection of the reserve functional capacity inherent in biological systems (Chisolm, 1971).

While research in other species has shown a reduction in the gamma globulin levels and antibody titers (De Bruin, 1971) this study did not demonstrate a significant change in any of the serum protein fraction percentages in the hamster. Lead is not the only heavy metal that has

been implicated as an immunosuppressent. Recent reports indicate that both cadmium and mercury caused a significant decrease in antibody titer (Koller, 1973). On this basis it appears that the phenomenon of antibody suppression is more closely related to species than it is to the presence or absence of lead. Hamsters would appear to be more highly resistant to the immunosuppressive effects of lead.

SUMMARY

One hundred and forty-four female golden Syrian hamsters, age 5 weeks, were apportioned into four treatment groups, with three replicates each, and subjected to two levels of lead exposure and two control treatments for 1, 2, and 3 weeks. They were subsequently challenged with a previously determined LD_{50} dose of Salmonella typhimurium. There were no statistically significant differences in mortality between the control and lead exposed animals. There were no significant differences in mortality among those animals exposed to lead for 1, 2, or 3 weeks nor was there an effect due to age at which the animals were first exposed to lead. The mean mortality for the exposure groups were: H_2O control, 69.4%; NaCl control, 57.3%; 125 mg/kg Pb, 63.9%; and 250 mg/kg Pb, 63.9%. The mean mortality for control and lead exposed groups for the three lengths of treatment exposure were: 1 week, 59.7%; 2 weeks, 62.5%; and 3 weeks, 68.8%. These means did not approach statistical significance.

Significantly higher blood lead levels were produced in the two lead exposed groups: H_2O control, 18 µg/100 ml; NaCl control, 15 µg/100 ml; 125 mg/kg Pb, 74 µg/100 ml; and 250 mg/kg Pb, 131 µg/100 ml. Blood lead levels had a high negative correlation with ALAD activity. Although the ALAD activity was drastically reduced only a minor reduction in hemoglobin concentrations occurred. There were no significant increases in red blood cells (RBC). It was concluded that the effects of reduced ALAD activity were not reflected by the hemoglobin concentrations and RBC counts.

There were significant increases in the hepatic and renal lead contents in the lead exposed groups. The mean hepatic tissue lead levels

were: H_2O control, 1.46 µg/gm; NaCl control, 1.38 µg/gm; 125 mg/kg Pb, 2.74 µg/gm; 250 mg/kg Pb, 4.75 µg/gm. The mean renal tissue lead levels were: H_2O control, 1.59 µg/gm; NaCl control, 1.89 µg/gm; 125 mg/kg Pb, 3.58 µg/gm; and 250 mg/kg Pb, 4.46 µg/gm. These increases were more dependent on the size of the daily dose than the age at initial exposure or length of exposure.

There were no significant differences between the control and lead exposed groups for each of the following parameters: percent mortality; times of death; total serum protein; serum albumin; $alpha_1$ globulin; $alpha_2$ globulin; beta globulin; gamma globulin; body weights; body weight gains; white blood cell counts; differential white blood cell counts; and red blood cell counts.

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