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REVIEW OF LEAD STUDIES IN ANIMALS CARRIED OUT AT HASKELL LABORATORY — TWO-YEAR FEEDING STUDY AND RESPONSE TO HEMORRHAGE STUDY

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ZUSAMMENFASSUNG

Es wird ein kurzer Überblick über zwei im Haskell-Laboratorium durchgeführte Tierversuche gegeben. Die erste Studie beschreibt eine zwei Jahre dauernde Ernährungsuntersuchung, während derer Ratten und Hunden Futter verabreicht wurde, dem 0, 10, 50, 100 *und* 500 ppm *Blei in Form von Blei-Azetat beigefügt worden war. Eine Rattengruppe erhielt überdies Dosen von* 1.000 *und* 2.000 ppm Pb. *An den Tieren wurden verschiedene klinische, biochemische, histopathologische und die Fortpflanzung betreffende Untersuchungen vorgenommen. In der zweiten Studie entnahm man Hunden mit äusserst niedriger* ALAD-*Tätigkeit grössere Blutmengen. Die Erholungskurven für Hämoglobin, die Zahl der roten Blutkörperchen und das Hämatokritverhältnis wurden mit denen der Kontrollhunde verglichen, denen die gleiche Blutmenge entnommen worden war.*

SUMMARY

A brief review of two animal studies carried out at Haskell Laboratory is given. The first describes a two-year feeding study during which rats and dogs were fed diets to which was added 0, 10, 50, 100 *and* 500 ppm *lead as lead acetate. Rats were also fed levels of* 1000 *and* 2000 ppm Pb. *Various clinical, biochemical, histopathologic and reproductive studies were done on the animals. In the second study, dogs with markedly depressed* ALA-*dehydrase activity were severely hemorrhaged. Their recovery curves of hemoglobin red cell count and hematocrit were compared to those obtained on hemorrhaged control dogs.*

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RÉSUMÉ

Présentation succincte de deux études effectuées sur des animaux au laboratoire Haskell. La première de ces études décrit une expérience portant sur une période de deux ans au cours de laquelle des rats et des chiens ont reçu une alimentation à laquelle étaient ajoutés 0, 10, 50, 100 *et* 500 ppm *de plomb sous forme d'acétate de plomb. Des rats ont également reçu une alimentation contenant* 1000 *et* 2000 ppm *de plomb. Différentes etudes cliniques, biochimiques, histopathologiques et des études de reproduction ont éte réalisées sur ces animaux. Dans la seconde étude on a pratiqué une saignee importante sur des chiens ayant une activité* ALA-D *nettement réduite. Les courbes de récupération de l'hémoglobine, du nombre de cellules rouges et de l'hématocrite ont été comparées avec celles obtenues sur des chiens de contrôle ayant également été saignés.*

1 — INTRODUCTION

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1 — INTRODUCTION

Today, I should like to summarize two animal studies carried out at Haskell Laboratory to provide additional information on the toxicology of lead. Both studies have been presented in more detail elsewhere [1, 2] and are only summarized here.

It has generally been agreed that in man, the diet is the major source of lead intake; however, long-term lead feeding studies in animals have only recently been carried out. Schroeder, *et al.* [3] reported that 5 ppm lead acetate in the drinking water of mice resulted in an increased mortality and decreased longevity. In a subsequent publication [4], however, these investigators reported that the water in the initial study published in 1964 contained 25 rather than 5 ppm lead and that the animals were chromium-deficient. When they repeated the study using non-chromium-deficient rats drinking water containing 25 ppm Pb, the mortality and longevity were not affected and lead was found to be non-tumorigenic. More recently, these investigators [5] have reported that 25 ppm Pb in the drinking water had a significant effect on the indices of reproduction of mice and rats.

Jessup [6] carried out one of the most comprehensive toxicologic studies of lead ever undertaken which included a 22-month feeding study using rats, dogs and monkeys, a three-generation reproduction study using rats, a teratology study in rabbits, a rat and monkey behavior study, a one-year newborn rat carcinogenesis study, tissue enzyme studies, electron microscopy and radiotracer studies. Ingestion of 10 ppm Pb produced no demonstrable adverse effects; 50 ppm resulted in minimal equivocal effects; and 100 and 1000 ppm produced histologic but not functional changes in the kidney of rats. Ten ppm Pb was equivalent to approximately 100 times the quantity of lead ingested by an average adult man.

2 — METHODS

In order to provide additional information on the effects of long term ingestion of lead, our laboratory fed diets containing lead acetate to rats and dogs for two years. The concentration of lead added was 0, 10, 50, 100 and 500 ppm. There were 50 male and 50 female rats per dose level and 100 male and 100 female control rats receiving the basal laboratory diet. Four male and four female beagle dogs were used at each dose level. After the study was in effect for several months, a second two-year feeding study was initiated in rats to provide dietary lead levels of 0, 1000 and 2000 ppm added lead. In the latter study, only 20 male and 20 female rats were used per dose level.

During the study, the clinical appearance and behavior of the animals was observed. A neurologic examination was done on the dogs by a recognized authority in veterinary neurology from the University of Pennsylvania. Food consumption, growth and mortality were recorded. Periodic blood, urine, fecal and tissue lead

analyses were done using atomic absorption spectrophotometry. A complete blood count, hemoglobin, hematocrit, stippled cell count, prothrombin time, alkaline phosphatase, urea nitrogen, glutamic-pyruvic transaminase, cholesterol, and albumin to globulin ratio were done periodically on the blood specimens. Routine and microscopic urinalyses were performed. The activity of the enzyme delta-aminolevulinic acid dehydrase (ALAD) in the blood and the excretion of its substrate, delta-aminolevulinic acid (DALA) in the urine were also determined.

A thorough necropsy including both gross and histologic examination was done on all animals that died, survived or were sacrificed during the two-year period. A three-generation, six-litter, reproduction study similar to that described by Oser and Oser [7] was done using the rats fed 0, 10, 50, 100, 1000 and 2000 ppm lead.

3 — RESULTS

There were no significant effects on the appearance, behavior, weight gain, mortality or neurologic examination of dogs receiving as much as 500 ppm Pb in their diet. The clinical appearance and behavior of the rats receiving as much as

TABLE 1

MORTALITY AND KIDNEY TUMORS IN RATS FED LEAD ACETATE FOR TWO YEARS

Dietary Pb[a] (ppm)	No. of Rats of Each Sex	% Mortality[b] Male	% Mortality[b] Female	% Kidney Tumors Male	% Kidney Tumors Female
5	100	37	34	0	0
18	50	36	30	0	0
62	50	36	28	0	0
141	50	[illegible]	28	0	0
548	50	52	36	10	0
5	20	50	35	0	0
1130	20	50	50	50	0
2102	20	80	35	80	35

a) Measured concentration of Pb in diet.

b) Includes rats that died or were sacrificed in extremis.

2000 ppm Pb was normal; however, the rate of weight gain was depressed in both male and female rats ingesting diets containing 1000 and 2000 ppm Pb. Table 1 shows that male rats fed 500 and 2000 ppm Pb had an increased mortality; however, the mortality at the 1000 ppm Pb level was not different from the control. The reason for this discrepancy is not known. It is also apparent from Table 1 that kidney tumors were seen in male rats at the 500 ppm Pb level and above. These were not seen in the females until the 2000 ppm Pb level. Most of the tumors were adenomas derived from the tubular epithelium. There were no pathologic changes seen in the rats fed up to and including 100 ppm lead in their diet. With the dogs, there were no

pathologic effects of dietary lead on any of the organ systems in the females. A slight degree of cytomegaly was found in the proximal convoluted tubule of the kidney in two of four male dogs fed 500 ppm Pb.

Table 2 shows the effect of dietary lead on the hematopoietic system. Hemoglobin and hematocrit were depressed only in the rats receiving 1000 ppm Pb or more.

TABLE 2

EFFECT OF DIETARY Pb ON HEMOPOIETIC SYSTEM
(Mean ± 95% C. L.[a])

Dietary Pb (ppm)	Hemoglobin (gm %)	Hematocrit (%)	Stippled Cells (cells/50 mg)	ALAD (units/ml rbc)	DALA (mg %)
5 Rat (2) (Dog)	15.39 (16.23)	42.9 (44.1)	0.05 (0.00)	22.0 (23.4)	0.42 (0.26)
18 (16)	15.27 (16.04)	42.2 (43.1)	0.18* (0.01)	22.2 (21.8)	0.43 (0.27)
62 (57)	15.42 (16.12)	42.3 (43.6)	0.33 (0.00)	14.4* (17.8)	0.42 (0.29)
141 (155)	15.32 (16.27)	42.1 (44.1)	0.50 (0.04)	9.3 (10.6)*	0.44 (0.27)
548 (576)	15.03 (15.64)	42.1 (42.4)	2.11 (0.23)*	3.6 (3.9)	0.97* (0.57)
± 95% C. L.	0.24 (0.57)	0.7 (1.8)	0.14 (0.11)	2.2 (4.1)	0.53 (0.08)
3	15.72	45.2	0.04	17.3	0.49
1130	14.73*	43.1*	4.27	2.4*	2.09*
2102	14.37	41.1	7.57	1.6	2.38
± 95% C. L.	0.29	0.8	0.63	0.7	0.21

a) C. L. = Confidence Limits. * Lowest level at which significant difference from control occurred ($p < .05$)

Stippled cells were increased at the 10 ppm level in the rat and were not increased in the dog until the diet contained 500 ppm Pb. A significant decrease in ALAD activity was seen at a dietary Pb concentration of 50 ppm in the rats and 100 ppm in the dogs. This decrease, however, was not associated with any increased urinary excretion of its substrate DALA until a concentration of 500 ppm Pb was present in the diet. These findings are consistent with the functional enzyme reserve concept seen in many biological systems [8]. No significant abnormalities were found in the other clinical chemistries examined.

Figure 1 shows that the blood Pb concentration of dogs rose rapidly during the first four to six months and, thereafter, appeared to plateau. Similar findings were found in the rats. Urine lead rose more promptly. The apparent lag time between continued ingestion of lead and subsequent rise in blood Pb may be a factor in the finding that pediatric lead poisoning cases accur more frequently in the summer months as opposed to the winter season when children would more likely be exposed to paint containing lead [9].

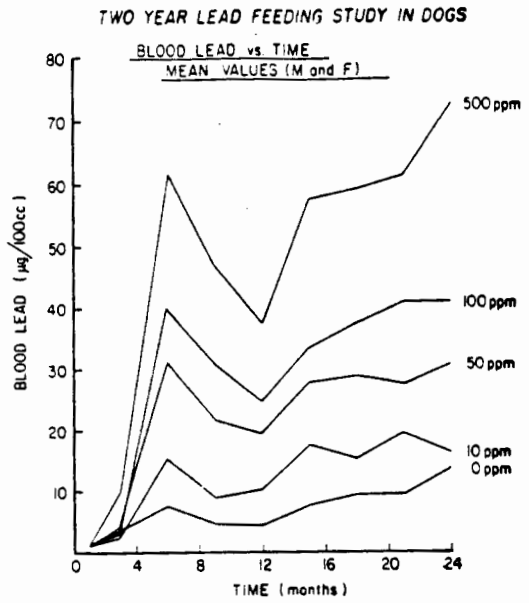

Fig. 1

A high degree of correlation was found between blood Pb concentration and concentrations of lead found in the kidney, bone, liver and brain of both rats and dogs. Figure 2 shows the high degree of correlation between blood Pb and kidney Pb concentration in dogs. Thus, blood Pb concentration appears to be a good index of tissue Pb deposition as well as exposure to Pb.

The three-generation, six-litter, reproduction study showed that there was no effect of dietary lead at concentrations of 0, 10, 50, 100, 1000 and 2000 ppm Pb on the number of pregnancies, number of pups born alive, fertility index, gestation index, viability index or lactation index. At 1000 and 2000 ppm dietary Pb, however, the average weight of weanling rats was slightly decreased and histologic examination of all organ systems in the 21-day old weanling rats (F_3B) showed histologic changes in the kidney comparable to those seen in adult rats receiving 500 ppm or more Pb in their diet (no tumors were seen).

The average concentration of lead in the blood, urine, kidney, liver, bone and brain of the rats and dogs after ingesting lead for 24 months is shown in Table 3. As the amount of lead in the diet was increased, the lead content of the tissues increased. In the dogs, the rate of increase was greatest for liver Pb and least for bone Pb. With rats, the rate of increase was greatest for the kidney rather than the liver. The blood and urine Pb concentration of dogs receiving 10 ppm Pb or more was significantly greater than corresponding control values. Similar findings occurred in the rat.

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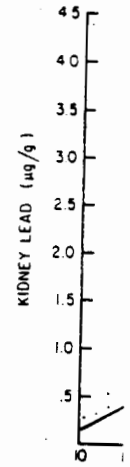

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The results from this study can be interpreted in several ways. On a weight per kilogram body weight basis, the dose levels are far above the daily dietary Pb intake of humans. For example, on a μg Pb/kg body weight basis, a 10 ppm Pb level in the rat is approximately 70 times higher than the average daily human dietary Pb intake.

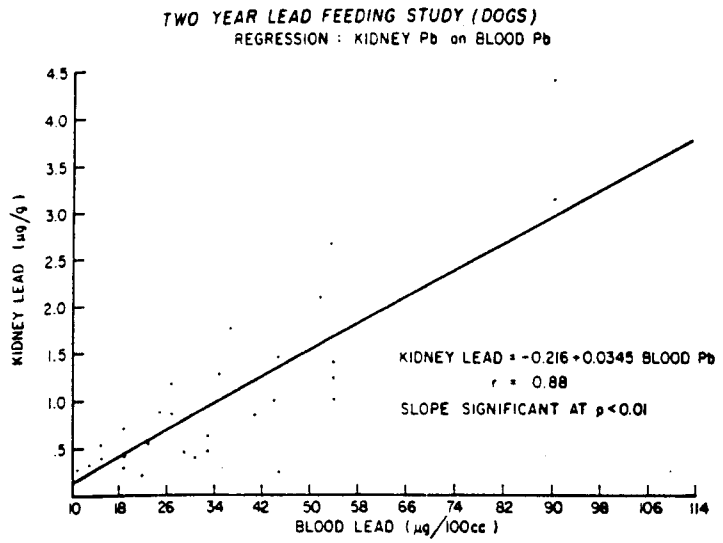


Fig. 2

On the other hand, the data in Table 3 suggest another possible interpretation. At concentrations of dietary Pb at or below 100 ppm, the blood and urine Pb concentrations, as well as the tissue levels, are comparable to normal human values. For example, normal human blood Pb concentrations are below 40 μg/100 gm. At these concentrations, the only significant finding reported is a decrease in ALA-dehydrase activity. In our study, the major finding in animals fed dietary Pb levels at or below 100 ppm for two years was inhibition of ALA-dehydrase. 100 ppm resulted in a blood Pb concentration of 31.5 in the dog and 35.2 in the rat and was not associated with any significant clinical, histopathologic, neurologic, reproductive, or mortality effects.

Similarly, adult humans generally begin to show clinical signs of lead poisoning at blood Pb values above 80 μg/100 gm. In this study, a dietary Pb level of 500 ppm produced a blood Pb concentration in both species of about 80 μg/100 ml which was associated with an increase in DALA excretion. At this level and higher, increased mortality and renal changes were observed in the rats. The latter may be attributable to the very high concentration of lead found in the kidney of the rat compared to man and the dog (Table 3).

Thus, when the results of this animal study are examined in terms of blood and tissue lead concentrations rather than on a basis of dose level, the findings are similar to those reported in humans.

TABLE 3

AVERAGE TISSUE LEAD CONCENTRATION IN RATS AND DOGS FED DIETS CONTAINING LEAD ACETATE

Dietary Pb (ppm)		Blood Pb[a] (µg/l)	Urine Pb[b] (µg/l)	Kidney Pb[a] (µg/g)	Liver Pb[a] (µg/g)	Brain Pb[a] (µg/g)	Bone Pb[a] (µg/g)
5	Rat	12.7	19	0.40	0.18	0.14	9.19
(1)	Dog	(15.8)	42	(0.22)	(0.50)	(0.05)	(4.88)
18		11.0	39*	0.31	0.16	0.19*	9.00
(16)		(16.6)*	42*	(0.41)*	(0.66)*	(0.06)	(5.19)
72		18.5*	79	0.85*	0.31*	0.28	17.50*
(57)		(31.5)	105	(0.74)	(1.57)	(0.98)*	(6.36)
141		35.2	148	1.51	0.60	0.38	26.75
(135)		(42.5)	156	(1.16)	(2.26)	(0.12)	(10.37)
548		77.8	451	15.20	1.87	1.06	91.40
(576)		(75.8)	329	(3.91)	(7.39)	(0.55)	(17.35)*
5		16.4	44	0.17	0.15	0.11	1.87
1130		38.6*	1042*	15.37*	1.87*	0.88*	280.54
2102		98.4	1281	11.60	2.86	1.48	409.05
Human[11,12]							
Mean		< 17	< 35	0.32	0.84	0.13	19.04
Range		15-40	20-65	.01-2.20	.03-3.13	.01-.78	.21-49.5

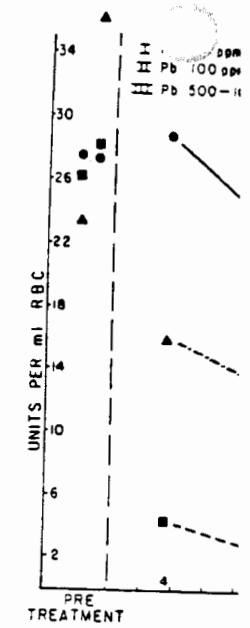
a = value at 24 months; b = average over 2-year period; * = lowest level at which significant ($p < .05$) difference from control occurred.

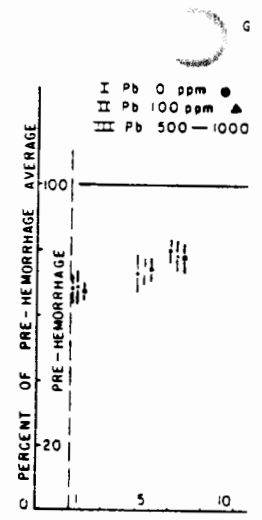
4 — HEMORRHAGE STUDY

ALA-dehydrase is one of the enzymes involved in hemoglobin synthesis. Our findings and those of others [8] suggest that despite a depression of its activity by lead, the body has sufficient enzyme reserve to meet its day to day needs. However, it could be argued that the body might not be able to cope with a stressful situation involving the loss of large quantities of blood. In order to investigate this situation, the following experiment was carried out.

Thirty-six dogs were divided into three groups of 12, with each containing six males and six females. One group served as a control; the second group received 100 ppm Pb as lead acetate in their diet and the third group was given 500 ppm Pb. After the animals were on this diet for 31 weeks, the dietary lead concentration of the high level group was doubled to 1000 ppm because the enzyme activity was not as low as desired.

Figure 3 shows that at the end of 46 weeks on this diet, the ALA-dehydrase activity of the dogs receiving the high level of lead was severely depressed. It varied from 0.5 unit/ml to barely detectable. When detectable, the enzyme level was about 2% of that prior to feeding lead. The group of dogs given 100 ppm Pb in the diet showed a 50% reduction in ALA-dehydrase activity. At the end of 46 weeks, the





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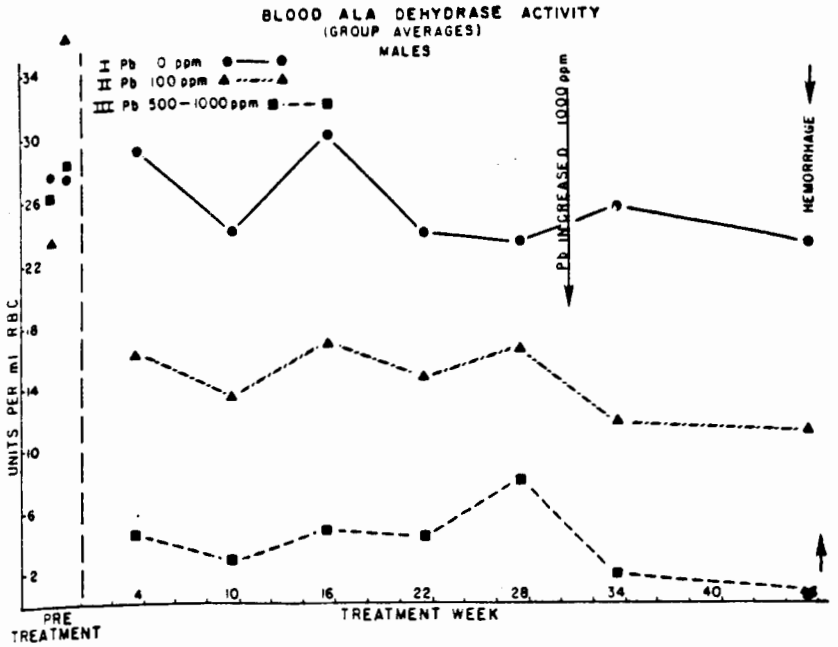

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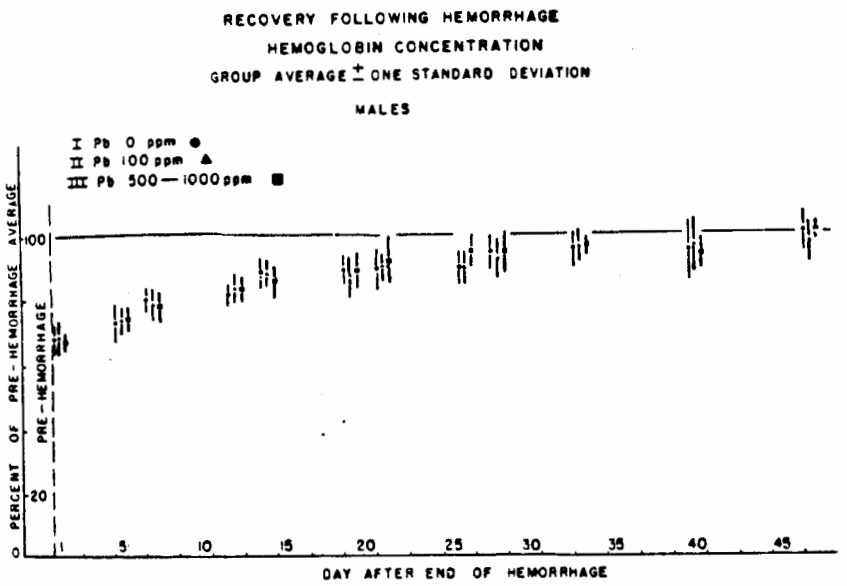

Fig. 4

blood concentration of the dogs given highest level of lead averaged 60-80 μg Pb/100 gm blood. There was no evidence of any difference in health of the dogs, in any of the groups, by any of the usual biochemical, clinical, or behavioral standards. Female dogs showed a significantly increased excretion of DALA.

Forty-six weeks after the dogs had been placed on these lead-containing diets, approximately one-half of the dogs circulating blood volume was removed from the jugular vein in a two-stage operation using a modified Whipple [10] procedure. Anesthesia was not necessary. Two of the 36 dogs showed definite signs of shock following the hemorrhage.

A 30 to 40 percent reduction in hemoglobin concentration, red cell count and hematocrit ratio occurred as a result of the hemorrhage. Figure 4 shows the recovery curve for hemoglobin concentration. Rigorous statistical treatment of the data demonstrated that the recovery curves of the hemoglobin concentration, red cell count and hematocrit ratio were not effected by the presence of lead.

This study clearly demonstrates that despite severe depression of ALA-debydrase activity, the ability of the blood-forming mechanisms to manufacture hemoglobin and red blood cells was not measurably affected even when required to junction at accelerated rates. It is reasonable to conclude that while ALAD is essential to the synthesis of hemoglobin, the amount of enzyme needed for this function is but a small fraction of that normally present.

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DISCUSSION

LEHNERT (Federal Republic of Germany)
In one of the diagrams which you showed, the blood lead level also rose in dogs with no lead burden during the observation period. How do you account for this phenomenon, or alternatively how can you substantiate this if the increase for a zero burden has been shown to be statistically impossible?

AZAR (U.S.A.)
The values shown on the slide were nominal. You will find the actual concentration of lead in the dirt of the control animals in the tables in the paper. The nominal concentrations represent the concentration of lead added to the diet which contained some lead.

BRIDBORD (U.S.A.)
Were animals that were bled continued on a lead diet after bleeding?

AZAR (U.S.A.)
Yes, they were continued on lead throughout the recovery period.

NEEDLEMAN (U.S.A.)
Did you attempt any refeeding of lead to offspring rats? Dr. Schroeder has reported dying out of the strain at the second generation when offspring rats were then put on low levels of lead intake.

AZAR (U.S.A.)
No; the reproduction studies of the F-2 generation were discontinued on the 21st day which was the weaning time. There were a few animals that were kept to the fortieth day and sacrificed then to look at the histopathological changes in their kidneys. There was a difference between the 21 day and 40 day weanlings; the histopathology seemed to be a little more pronounced, but we did not continue the F-2 generations beyond that time.

NEEDLEMAN (U.S.A.)
I think this refeeding experiment may have important implications for human newborns exposed in utero, and then reexposed to lead in childhood.

DUBOIS (Canada)
A conclusion from this study seems to be that it is difficult to affect the ALAD levels by the ingestion of large amounts of lead in food. Would the author care to comment as to whether the ALAD levels would have been affected if similar amounts of lead had been administered in aerosol form by the respiratory route?

AZAR (U.S.A.)
The ALAD levels were affected by the ingestion of large amounts of lead in the food. Similar amounts of lead by the respiratory route would also be expected to lower the level of ALAD. Experimental human exposures to 10.9 $\mu g/m^3$ lead resulted in a depressed level of ALAD which recovered following exposure (Coulston *et al.*, Albany Medical College, Albany, New York).

KENNEDY Jr. (U.S.A.)

One of the concerns to toxicologists is the increased excretion of heme precursors following inhibition of the biosynthetic enzymes by lead. Urinary excretion of ALA indicates an oversupply and spill-over situation. The biologic consequences of increased circulating ALA were studied by our group in mice. Graded doses (10 to 100 mg/kg) were introduced directly into the peritoneal cavity three times per week for 13 consecutive weeks. During the treatment period, all animals were monitored as to growth, reactivity and blood chemistry. No adverse effects upon any of the parameters studied were observed and histopathological examination of tissues and organs was also negative.

In addition, a study of mice treated chemically with ALA indicated that no interference with normal fertility was induced by the chemical.