1849-16

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

Alex Azar, M.D., Henry J. Trochimowicz, D.Sc.,

Mary E. Maxfield, Ph.D.

E. I. du Pont de Nemours and Company

Haskell Laboratory

Elkton Road

Newark, Delaware 19711

N40702

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.

DUP050057949

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

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.

METHODS

In order to provide additional information on the effects of long-term ingestion of lead, our laboratory fed diets containing lead acetate to [redacted] 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.

DUP050057950

During
animals was
by a recogni
of Pennsylva
Periodic blo
atomic absor
hematocrit,
urea nitrog
to globulin
Routine and
enzyme delt
excretion o
urine were

A thor
was done on
the two-yea

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.

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 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. ~~The females showed 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.~~ 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 II~~ 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. 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.

DUP050057951

Next Slide

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 and with urine lead. 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 occur more frequently in the summer months as opposed to the winter season when children would more likely be exposed to paint containing lead.(9)

Next Slide

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.

Next Slide

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~~ of 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 ~~[illegible]~~ cytomegaly, intranuclear inclusions, karyolysis and hyperplasia.

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 III. 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.

On the other hand, the data on this slide suggest another possible interpretation. For example, normal human blood Pb concentrations are generally below 40 ug/100 gm and at these concentrations the only significant finding has been a decrease in ALA dehydrase activity. In our study, the animals fed 100 ppm Pb in their diet had blood and tissue Pb concentrations comparable to normal humans and the major finding in these animals was a decrease in ALA-dehydrase activity. This decrease in ALA-dehydrase activity was not associated with any significant clinical, histopathologic, microscopic, reproductive or mortality effects.

ONLY TELL THOSE WHO HAVE A NEED TO KNOW

DUP050057952

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 III).

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.

HEMORRHAGE STUDY

ALA-dehydrase is one of the enzymes involved in hemoglobin systhesis. 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 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 and only Two of the 36 dogs showed definite signs of shock following the hemorrhage.

DUP050057953

A 30 to 40 percent reduction in hemoglobin concentration, red cell count and hematocrit ratio occurred as a result of the hemorrhage. ~~Figure 1~~ Table I 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-dehydrase activity, the ability of the blood-forming mechanisms to manufacture hemoglobin and red clood cells was not measurably affected even when required to function 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.

DUP050057954

REFERENCES

1. Trochimowicz, H. J., J. R. Barnes, H. Sherman, E. F. Stula and N. W. Henry: The Effect of Prolonged Feeding of Low Levels of Lead to Laboratory Animals. Presented at the American Industrial Hygiene Association Meeting, San Francisco, California, May 1972. Paper will be submitted to the American Industrial Hygiene Journal.

2. Maxfield, M. E., G. J. Stopps, J. R. Barnes, R. D. Snee and A. Azar: Effect of Lead on Blood Regeneration Following Acute Hemorrhage in Dogs. American Industrial Hygiene Association Journal (May 1972).

3. Schroeder, H. A., J. J. Balassa and W. H. Vintom: Chromium, Lead, Cadmium, Nickel and Titanium in Mice: Effect on Mortality, Tumors and Tissue Levels. Journal of Nutrition, 83:239-250 (July 1964).

4. Schroeder, H. A., M. Mitchener and A. P. Nason: Zirconium, Niobium, Antimony, Vanadium and Lead in Rats: Life-Term Studies. Journal of Nutrition, 100:59-68 (1970).

5. Schroeder, H. A. and M. Mitchener: Toxic Effects of Trace Metals on the Reproduction of Mice and Rats. Archives of Environmental Health, 23:102-106 (1971).

6. Jessup, D. C.: The Chronic Toxicity of Lead, American Petroleum Institute Medical Research Report No. EA7102. The Clearinghouse House for Federal Scientific and Technical Information, National Technical Information Services, 5285 Port Royal Road, Springfield, Virginia 22151 (April 1971).

7. Oser, B. L. and M. Oser: Nutritional Studies on Rats on Diets Containing High Levels of Partial Ester Emulsifiers, Journal of Nutrition, 60:4, pp. 489-505 (December 1956).

8. Lead, Airborne Lead in Perspective, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D. C. 20418 (1972).

9. Chisolm, J. J., Jr.: Lead Poisoning, Scientific American, 224:15-23 (1971).

10. Whipple, G. H., C. W. Hooper and F. S. Robschert: Blood Regeneration Following Simple Anemia, I, Mix Diet Reaction, American Journal of Physiology, 53(2):151 (September 1920).

11. Barry, P.S.I. and Mossman, D. B.: Lead Concentrations in Human Tissues. Brit. J. Industr. Med. 27:339-351 (1970).

12. Goldwater, L. J. and Hoover, A. W.: An International Study of "Normal" Levels of Lead in Blood and Urine. Arch. Environ. Health 15:60-63, (1967).

DUP050057955

TABLE I

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	Female	% Kidney Tumors Male	Female
5	100	37	34	0	0
18	50	36	30	0	0
62	50	36	28	0	0
141	50	36	28	0	0
548	50	52	36	10	0
3	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.

TABLE II

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	15.39	42.9	0.05	22.0	0.42
(2) (Dog)	(16.23)	(44.1)	(0.00)	(23.4)	(0.26)
18	15.27	42.2	0.18*	22.2	0.43
(16)	(16.04)	(43.1)	(0.01)	(21.8)	(0.27)
62	15.42	42.3	0.33	14.4*	0.42
(57)	(16.12)	(43.6)	(0.00)	(17.8)	(0.29)
141	15.32	42.1	0.50	9.3	0.44
(155)	(16.27)	(44.1)	(0.04)	(10.6)	(0.27)
548	15.03	42.1	2.11	3.6	0.97*
(576)	(15.64)	(42.4)	(0.23)*	(3.9)	(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 ($p < .05$)

TABLE III

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

Dietary Pb (ppm)	Blood Pb[a] (ug%)	Urine Pb[b] (ug/l)	Kidney Pb[a] (ug/g)	Liver Pb[a] (ug/g)	Brain Pb[a] (ug/g)	Bone Pb[a] (ug/g)
5 Rat	12.7	19	0.40	0.18	0.14	9.19
(2) Dog	(15.8)	42	(0.22)	(0.30)	(0.05)	(4.88)
18	11.0	39*	0.31	0.16	0.19*	9.00
(16)	(16.6)*	62*	(0.41)*	(0.66)*	(0.06)	(6.19)
62	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	2.31	0.60	0.38	26.75
(155)	(42.5)	156	(1.16)	(2.26)	(0.12)	(10.37)
548	77.8	451	13.20	1.87	1.06	91.40
(576)	(75.8)	325	(2.91)	(7.89)	(0.55)	(17.35)*
3	16.4	44	0.17	0.13	0.11	1.87
1130	98.6*	1046*	13.37*	1.87*	0.88*	280.54
2102	98.4	1281	11.60	2.86	1.48	409.05
Human[11,12]						
Mean	< 17	< 35	0.52	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.

DUP050057956

FIGURE 1

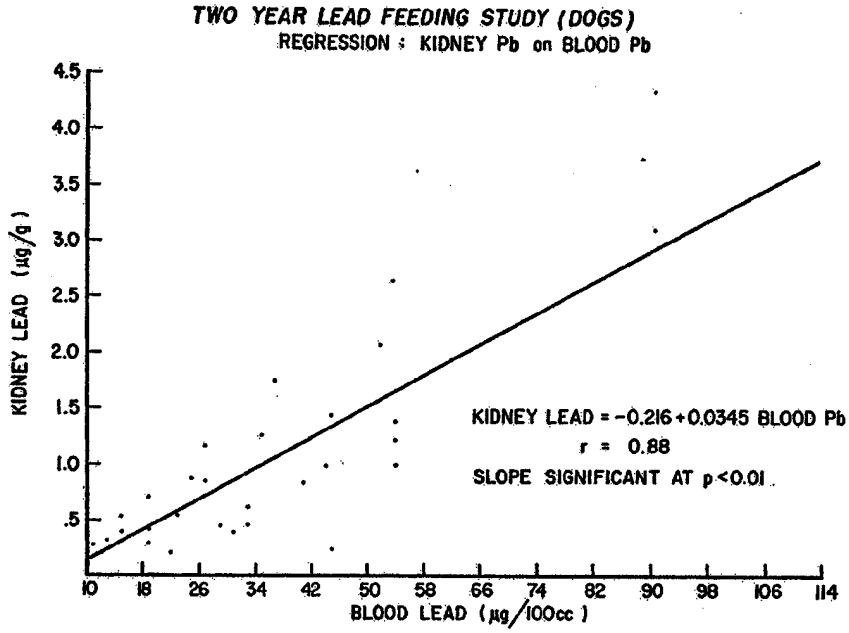
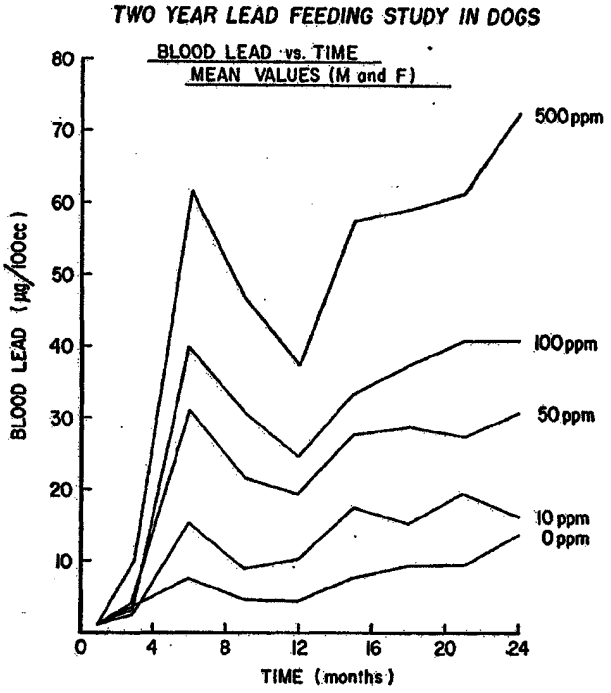


FIGURE 2



DUP050057957

FIGURE 3

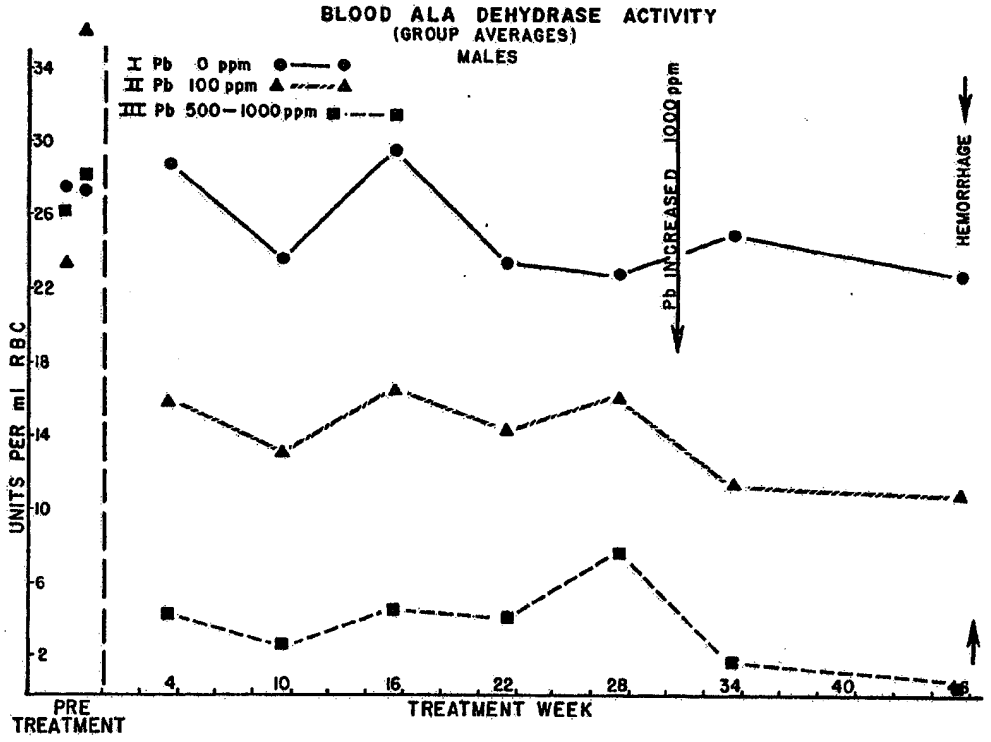
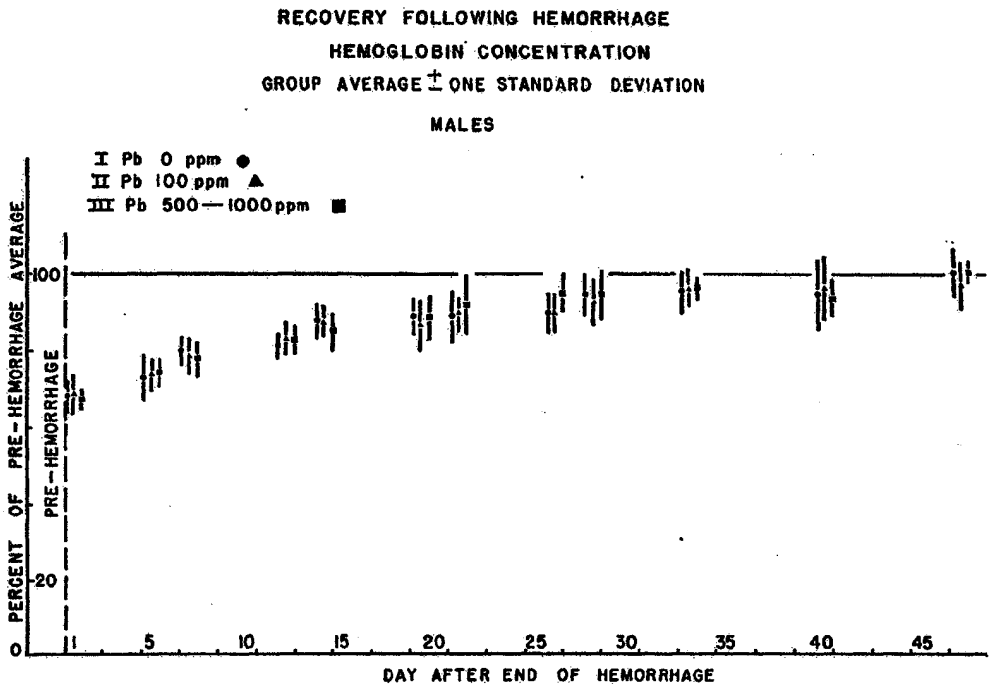


FIGURE 4



DUP050057958