Medical Research Project 787

HEMATOLOGIC AND BIOCHEMICAL RESPONSES OF DOGS TO LEAD ACETATE IN THEIR DIET

PROCEDURE:

Samples of blood and urine were collected three times before the test began, after one, two and three months and then every three months for the remainder of the two years. Blood was taken by venipuncture for the hematologic and biochemical measurements. A 24-hour urine specimen was collected on the day preceeding or following the blood sample. Two dogs, one male and one female, were sacrificed at the end of one year. All but one female survived the second year. At the end of the test period, the remaining animals were sacrificed and tissues were taken for lead analysis.

METHODS:

The hematologic examination of the blood, made on a portion of the sample collected, was the same as described for the rats.

The urinalysis consisted of a measure of the specific gravity, a semi-quantitative measure of urobilinogen[1] and a test for acetone[2] and bilirubin[3] as well as the routine analyses described for the rats. Urine glutamic-pyruvic transaminase, however, was not measured.

The biochemical measurements made on the blood included a measure of: glucose using Boehinger reagent[4]; urea nitrogen by the method of Karr[5]; cholesterol by the method of Pearson, Stine and McGarack[6]; alkaline phosphatase by a modification of the method of Bessey, Lowry and Brock using Boehinger reagent[7]; glutamic-pyruvic transaminase by the method of Wroblewski and LaDue[8].

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1. Watson, C.J., Am. J. Clin. Path., 6:458 (1936).
2. Acetest®, Ames Co., Elkhart, Indiana.
3. Ictotest®, Ames Co., Elkhart, Indiana.
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5. Karr, W.G., Manual of Clin. Biochem., Stephenson Bros., Phila (1940).
6. Pearson, S., S. Stein and T.H. McGarack, Anal. Chem., 25:881 (1953).
7. Bessey, O.A., O.H. Lowry and M.S. Brock, J. Biol. Chem., 164:321 (1946).
8. Wroblewski, F. and J. S. LaDue, Proc. Soc. Expt'l. Biol. Med., 91:569 (1956).

METHODS (Cont'd.):

Delta-aminolevulinic dehydrase was also measured after 6, 9, 12, 15, 18 and 24 months using the method of Bonsignore, et al. [9]. Prothrombin time was measured using the Lab-Tek Prothrombin system[10] and the total protein with the buiret reagent[11]. Albumin globulin ratios were determined after electrophoretic separation of the proteins using the dye elution technique[12].

Samples of blood were taken for lead analysis and stored in plastic bottles at -4° C until analyzed. Urine and feces samples were collected as described previously for rats. Tissues from individual animals were collected at the terminal sacrifice and kept frozen until analyzed. Lead was determined by atomic absorption spectrophotometry following the same procedure described for the samples from rats.

A simple correlation coefficient was calculated for the response vs. lead in the diet for each of the quantitative measurements made on the blood, urine, feces or tissue. The coefficient of regression with time was also calculated for these responses. An analysis of variance was made on the data for those responses that showed a significant correlation with the lead level of the diet and a "t" test of the difference between means was used to determine the effect level.

RESULTS:

The average value found for each response measured during the pre-exposure period and for the test period, when lead was added to the diet, are listed in Table IV. The simple correlation coefficient for the response vs. lead content of the diet also appears in this table. The group averages at the various dietary levels of lead are listed in Table V for males and in Table VI for the females. Averages for each sampling interval during the two-year period are found in the appendix.

The correlation coefficient indicated that there was a very significant relationship between the lead in the diet and the delta-aminolevulinic acid in the urine, the aminolevulinic dehydrase activity of the blood, and the lead content of the urine, feces and blood. A similar, but much less definite relationship appeared to exist for several other responses that are of interest because of their association with lead intoxication.

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9. Bonsignore, D., P. Calissano, and C. Cortasegud, Bul. Hygiene 40:1337 (1965).
10. Lab-Tek Instrument Co., Westmont, Ill.
11. Reiner, M., Standard Methods of Clinical Chemistry, Vol. I, Academic Press, New York (1953).
12. Gelman Instrument Co., Ann Arbor, Michigan, Manual 51199-A (1963).

RESULTS (Cont'd.):

A. delta-Aminolevulinic Dehydrase:

The enzyme, delta-aminolevulinic dehydrase (ALD) is involved in the early stages of heme synthesis. Two molecules of aminolevulinic acid are condensed to form porphobilinogen by the catalytic action of this enzyme in the biological system. Lead has been shown to inhibit ALD activity both in vivo and in vitro, and its activity has been reported to be one of the most sensitive indications of an exposure to lead.

The simple correlation coefficient indicated that the decrease in ALD activity with increasing levels of lead in the diet were definitely related. The "t" test of the difference between means showed that the addition of 50 ppm or more of lead to the diet of the dogs resulted in a significant decrease in ALD activity in the blood.

B. delta-Aminolevulinic Acid:

Although the increase in the concentration of delta-aminolevulinic acid (DALA) in the urine with increasing levels of lead in the diet was not very marked at the lower lead supplements, the correlation coefficient indicated a significant relationship. The DALA in the urine of dogs receiving up to 100 ppm of added lead in their diet, however, did not differ significantly from the dogs receiving no added lead. A "t" test of the difference between means showed that only the dogs receiving 500 ppm of added lead had elevated levels of DALA in their urine.

C. Other Changes in the Blood:

A small, but nevertheless, significant relationship between the lead in the diet and the hemoglobin concentration and hematocrit was also indicated by the simple correlation coefficient. There was no marked difference between the average value observed for any group for these responses. The mean value for all dogs during the two-year period was actually somewhat higher than the average value measured during the pre-test period. In view of the demonstrated effect on heme synthesis, however, the results might be considered to suggest the beginning of a trend toward abnormal at the highest lead supplement to the diet.

Less significant, but noteworthy, was the slight decrease in alkaline phosphatase activity that showed some correlation with the lead added to the diet, for such a response also occurred in rats fed these lead supplements. The transaminase activity and dietary lead showed a similar relationship and suggests the effect may have resulted from simple heavy metal inhibition of enzyme activity rather than reflecting an effect on a tissue at the levels of lead that were added to the diet.

RESULTS (Cont'd.):

D. Lead in Blood:

During the pre-test period and for the first two months of the test, it was not possible to detect or measure lead in the blood with the sample available. After that time, however, the analytical procedure was modified to increase its sensitivity and lead was measured in all subsequent samples.

The correlation coefficient showed a very marked relationship between the lead concentration in the blood and the lead in the diet. At the various levels of lead added to the diet in this study, the males usually had higher levels of lead in the blood than the females. The length of time that lead was fed also influenced the lead level in the blood for there was a trend toward higher values as the experiment progressed.

The difference between the mean blood lead level of the group receiving no added lead in the diet and the mean of the group receiving a 10 ppm lead supplement was significant (sexes tested separately). The mean blood-lead concentration for the control group (males and females) was 0.8 µg/10 ml ± 0.6 µg/10 ml. If the upper range of a normal population is taken to be plus two, standard deviations from the mean, only 24% of the measurements in the group fed 10 ppm of added lead were outside this range. The chance of detecting an exposure to 10 ppm of lead in the diet by measuring blood lead would have been one time in four. Even at the highest lead supplement (500 ppm) nearly 9% of the values were in the "normal" range (i.e. less than +2 S.D. from the control mean). For the control population itself, approximately 3% of the values were above this "normal" range.

The control group mean of 0.8 µg/10 ml is somewhat less than half the value 1.7 µg/10 ml ± 1.1 µg/10 ml (17 ± 11 µg/100 ml) reported by Goldwater and Hoover[13] for "normal" blood levels in humans. The dogs receiving the 10 ppm lead supplement more closely approximated this value. A level of 8 µg/10 ml (80 µg/100 ml) according to Haeger-Aronson[14] has usually been given as the upper normal limit of the concentration of lead in the blood of humans. Values exceeding this range were seen only in the dogs receiving the highest lead supplement approximately 20% of the time.

E. Lead in Urine and Feces:

There was a very significant correlation between the level of lead in the urine and feces and the level of lead in the diet. Most of the lead entering the human body by ingestion is eliminated by way of the intestines without being absorbed into the blood stream[13]. Since this is probably true for other mammalian species as well, only the urinary lead data, representing the absorbed lead, was analyzed statistically.

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13. Goldwater, L. J. and A. W. Hoover, Arch. Environ. Health, 15:64 (1967).
14. Haeger-Aronson, B., Second. J. Clin. & Lab. Invest. 12: Supplement 47 (1966).

RESULTS (Cont'd.):

The amount of lead eliminated by the kidneys did not change significantly as the study progressed nor did one sex have, consistantly, higher or lower urinary lead levels than the other. The difference between the mean level of urinary lead of the group receiving the 10 ppm lead supplement and the control group with no added dietary lead was significant. The mean urine lead concentration of the control group (male plus female) was 0.41 µg/10 ml ± 0.26 µg/ml. Only 13% of the urine lead concentrations in the group fed the 10 ppm supplement were outside the normal range, if plus two standard deviations from the control mean is used to establish the upper limit of normal. The chance of detecting an exposure to an increase of 10 ppm of lead in the diet by measuring urinary lead would be about 1 time in 8. At the highest dietary lead supplement somewhat less than 2% of the urine lead concentrations were within the normal range established by the controls.

The control group mean of 0.41 µg/10 ml is about 25% higher than the value 0.33 µg/10 ml (33 µg/L) reported by Goldwater and Hoover for "normal" lead levels in human urine. The upper normal limit of human urinary lead given by most authors according to Haeger-Aronson is 0.6 - 0.8 µg/10 ml (6.0 - 8.0 µg/100 ml). The mean for the group, males and females, receiving the 10 ppm lead supplement was in this range and 40% of the individual analyses for this group gave results which were in excess of 0.6 µg/10 ml.

F. Lead in Tissues:

The results of the lead analyses of the various tissues collected at the termination of the study are summarized in Table VII. Since the number of samples at each level of dietary lead was quite small all of the statistical techniques could not be used to analyze the data.

A simple correlation coefficient indicated that for all but the muscle there was a significant relationship between the lead content of the tissue and the lead in the diet. For those tissues whose lead content was related to the lead in the diet, the highest concentration was found in the bone and the lowest in the brain. There was, in general, a similar relative increase in tissue lead with increasing amounts of lead in the diet indicated by the slope for the transformed data, log x on log y, although the rate of increase was somewhat greater for the liver and somewhat lower for the bone than for the other tissues. (Figure 4)

COMMENTS:

The significant responses in rats and dogs fed lead in their diet are summarized in Table VIII for comparison.

Paul

The average lead level in the diet of the rats during the two-year period was higher than in the diet of the dogs. Some of this difference resulted from variation in the lead content of the basal diet and some undoubtedly resulted from errors in the mixing, sampling and analyses for lead. The average fecal lead content was also higher in the rats than in the dogs.

COMMENTS (Cont'd.):

The urine lead concentration, expressed both as µg/10 ml and as a per cent of the lead ingested was higher in the dogs than in the rats. The significance of this observation is not certain. It may represent a species difference in the absorption of dietary lead, a difference in the route of elimination, a difference in the retention and storage of lead or a combination of these and other factors.

The biochemical evidence for an effect of added lead in the diet, an increase in urinary DALA reflecting an interference with heme synthesis and an increase in stippled cells reflecting a greater incidence of defective erythrocytes would suggest that the rat is the more sensitive species.

Higher concentrations of lead were found in the bone, kidney and brain of the rat than the dog. In the blood and liver higher concentrations of lead were found in the dog than the rat. Because of the association of bone with erythrocyte production, hence indirectly with heme synthesis, the greater accumulation of lead in the bone of rats may account for the species difference in sensitivity to ingested lead.

Aside from possible or probable species difference in absorption, transport, accumulation or elimination of dietary lead, there were important differences in the treatment of each species that may have more bearing on the apparent difference in sensitivity. The rats began receiving the dietary lead when they were weaned and continued to receive it for essentially their lifetime. The dogs were young adults when lead was added to their diet and were fed for a portion of their lifetime. As a consequence of this, the rats received a higher relative dose--grams lead/grams body weight when they were young and perhaps more vulnerable than they did when they were mature. For the dogs there was little change in the relative dose throughout the feeding period.

The level of lead in the tissues and in the blood, urine and feces was directly related to the lead content of the diet. An increase in the lead content of any of these may be considered, per se, an effect or response. In the blood and excretia, such an effect was discernible even at the lowest dietary lead supplement. A response observed in both rats and dogs that can be considered an adverse effect in that it is evidence for an interference with a necessary biochemical process was found for the synthesis of heme. This occurred only at the highest dietary level of lead. A similar kind of effect was also found at this level of lead for erythrocyte development in rats.

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TABLE 4
SIMPLE CORRELATION COEFFICIENT OF RESPONSE TO LEAD IN THE DIET
TWO YEAR FEEDING STUDY WITH DOGS

	Mean		Coefficient of Correlation		
	Control	Test		Males	Females
Erythrocytes, $X10^6/mm^3$	5.202	5.557	-0.056	0.091	-0.185
Hemoglobin, gm/100 ml	16.00	16.03	-0.318	-0.095	-0.520
Hematocrit, %	42.9	43.5	-0.313	-0.151	-0.474
Leucocytes, $X10^3/mm^3$	15.84	14.18	-0.090	-0.284	0.029
Neutrophils, Segmented, %	52.2	53.7	0.046	0.312	-0.193
Neutrophils, Juvinile, %	4.9	1.9	-0.076	0.063	-0.076
Myelocytes, %	0.4	0.4	-0.140	0.038	-0.140
Lymphocytes, %	30.9	28.9	0.024	-0.114	0.130
Eosinophils, %	7.8	9.3	-0.132	-0.455	0.270
Monocytes, %	3.1	4.6	0.108	0.255	-0.126
Basophils, %	0.2	0.3	-0.012	-0.308	0.048
Atypical Cells, %	0.5	0.8	-0.140	-0.180	-0.118
Nucleated RBC'S/100 WBC'S	0.1	0.2	-0.032	-0.117	0.061
Stippled Cells/50 mf	0	0.05	-	-	-
Urine Volume, ml/24 hrs.	329	482	-0.238	-0.410	-0.070
Specific Gravity	1.0312	1.0266	0.121	0.129	0.114
Osmolality, mOs/L	1205	864	0.000	0.082	-0.067
Protein, mg/24 hrs.	14.21	21.95	0.096	0.197	-0.058
δ-Aminolevulinic Acid, mg/24 hrs.	0.66	1.40	0.657	0.720	0.589
Glucose, mg %	71.0	59.6	-0.078	-0.334	0.149
Urea Nitrogen, mg %	15.24	14.17	0.191	0.226	0.151
Cholesterol, mg %	125.4	133.4	0.047	0.010	-0.090
Alkaline Phosphatase, Bessey Units	1.47	1.35	-0.204	-0.107	-0.290
Transaminase (GPT), units	13.3	18.7	-0.246	-0.288	-0.244
Prothrombin Time, sec.	10.0	9.8	-0.177	-0.341	-0.126
Total Protein, gm/100 ml	5.731	6.08	0.227	-0.084	0.472
Albumin/Globulin	1.151	1.175	-0.084	-0.032	-0.564
δ-Aminolevulinic Acid Dehydrase		15.56	-0.824	-0.832	-0.828
Lead in Urine, μg/10 ml	-	1.36	0.834	0.809	0.867
Lead in Feces, μg/gm.	-	342	0.873	0.896	0.863
Lead in Blood, μg/10 ml	-	2.61	0.841		

TABLE 5
AVERAGE VALUE FOUND FOR THE MEASUREMENTS MADE ON BLOOD AND URINE OF MALE DOGS FED LEAD ACETATE FOR TWO YEARS

	Pre-Test			I	II	III	IV	V
	N	$\overline{X}$	± S.D.	milligrams LEAD/gram FEED				
				2	16	57	155	576
Erythrocytes, $X10^6/mm^3$	59	5.28	0.62	5.49	5.38	5.58	5.92	5.50
Hemoglobin, gm/100 ml	59	15.9	1.7	16.1	16.1	15.8	16.7	16.0
Hematocrit, %	59	43.0	3.9	43.5	43.7	43.0	46.2	43.0
Leucocytes, $X10^3/mm^3$	59	16.56	4.1	14.17	16.57	15.47	12.72	13.97
Neutrophils, Segmented %	58	51.8	8.2	53.5	54.7	55.5	51.0	57.7
Neutrophils, Juvinile %	58	5.9	2.6	2.5	2.1	2.3	2.3	2.8
Myelocytes, %	58	0.4	0.6	0.6	0.3	0.5	0.3	0.5
Lymphocytes, %	58	29.1	7.6	26.9	26.7	29.0	28.2	26.4
Eosinophils, %	58	9.0	6.6	11.1	10.7	7.4	13.0	5.6
Monocytes, %	58	3.0	1.8	4.2	5.2	4.7	4.9	6.1
Basophils, %	58	0.1	0.6	0.5	0.3	0.3	0.3	0.1
Atypical Cells, %	58	0.6	1.1	1.0	0.7	0.6	0.7	0.6
Nucleated RBC'S/100 WBC'S	58	0.2	0.5	0.5	0.1	0.2	0.2	0.2
Stippled Cells/50 mf	58	0	0	0	0	0	0.03	0.20
Urine Volume, ml/24 hrs.	60	349	184	579	497	565	395	432
Specific Gravity	60	1.031	0.009	1.025	1.027	1.022	1.032	1.027
Osmolality, mOs/L	60	1205	361	819	876	716	1040	851
Protein, mg/24 hrs.	60	20.5	26.2	27.9	28.8	35.4	19.0	42.2
delta-Aminolevulinic Acid, mg/24 hrs.	60	0.75	0.47	1.30	1.28	1.04	1.10	2.36
Alkaline Phosphatase, μM pNP/ml/hr.	59	1.46	0.2	1.21	1.45	1.30	1.40	1.25
Glutamic-Pyruvic Transaminase, units	60	15	8	19	23	24	23	17
Glucose, mg %	59	75	9	61	60	63	57	57
Urea Nitrogen, mg %	59	15.2	3.5	14.5	13.8	13.6	14.9	15.7
Cholesterol, mg %	59	118	19	119	124	140	121	135
Total Protein, gm/100 ml	60	5.77	0.45	6.09	6.45	6.07	5.84	6.12
Albumin/Globulin	60	1.15	0.20	1.11	0.99	1.11	1.16	1.18
Prothrombin Time, sec.	20	9.7	-	9.3	9.6	9.7	9.5	8.9
delta-Aminolevulinic Acid dehydrase, units/ml	-	-	-	25	25	16	12	4

TABLE 6

AVERAGE VALUE FOUND FOR THE MEASUREMENTS MADE ON BLOOD AND URINE OF FEMALE DOGS FED LEAD ACETATE FOR TWO YEARS

	Pre-Test			I	II	III	IV	V
	N	$\bar{X}$	± S.D.	milligrams LEAD/gram FEED				
				2	16	57	155	576
Erythrocytes, $X10^6/mm^3$	60	5.21	0.52	5.61	5.28	5.41	5.32	5.06
Hemoglobin, gm/100 ml	60	16.1	1.3	16.5	16.0	16.5	15.9	15.3
Hematocrit, %	60	43.5	3.7	45.0	42.8	44.5	43.3	40.3
Leucocytes, $X10^3/mm^3$	60	15.40	3.9	13.15	13.92	14.10	14.60	14.35
Neutrophils, Segmented %	59	52.6	9.5	55.5	52.2	53.2	52.0	53.0
Neutrophils, Juvinile %	59	4.0	2.0	2.0	2.2	2.0	1.8	2.0
Myelocytes, %	59	0.3	0.6	0.4	0.5	0.3	0.5	0.2
Lymphocytes, %	59	32.7	8.0	27.6	29.1	30.6	33.6	30.4
Eosinophils, %	59	6.7	3.8	8.8	10.1	8.3	6.7	11.2
Monocytes, %	59	3.2	1.9	4.5	4.1	4.6	4.3	4.0
Basophils, %	59	0.2	0.6	0.4	0.6	0.3	0.2	0.5
Atypical Cells, %	59	0.3	0.6	1.0	1.4	0.8	0.6	0.9
Nucleated RBC'S/100 WBC'S	59	0.1	0.3	0.2	0.2	0.2	0.2	0.2
Stippled Cells/50 mf	59	0	-	0	0.03	0	0.05	0.15
Urine Volume, ml/24 hrs.	60	309	168	421	538	405	553	437
Specific Gravity	60	1.0032	0.011	1.026	1.023	1.034	1.022	1.029
Osmolality, mOs/L	60	1205	462	881	770	1132	692	868
Protein, mg/24 hrs.	60	7.9	9.5	9.6	13.9	13.8	18.4	10.5
delta-Aminolevulinic Acid mg/24 hrs.	59	0.57	0.33	1.18	1.19	1.28	1.24	2.00
Alkaline Phosphatase μM pNP/ml/hr.	60	1.51	0.6	1.48	1.28	1.43	1.48	1.18
Glutamic-Pyruvic Transaminase, units	60	12	3	15	18	16	20	13
Glucose, mg %	60	71	12	58	58	58	64	60
Urea Nitrogen, mg %	60	15.9	3.5	13.0	13.4	14.4	14.1	14.3
Cholesterol, mg %	60	135	28	146	156	125	129	139
Total Protein, gm/100 ml	60	5.68	0.57	6.03	5.97	6.00	5.67	6.63
Albumin/Globulin	60	1.15	0.28	1.18	1.09	1.16	1.18	0.86
Prothrombin Time, sec.	20	10.4	2	8.2	9.3	9.6	12.8	9.3
delta-Aminolevulinic Acid dehydrase, units/ml	-	-	-	22	19	20	9	4

TABLE 7

AVERAGE VALUE FOUND FOR THE LEAD CONTENT OF TISSUES FROM DOGS FED LEAD ACETATE FOR TWO YEARS

	I	II	III	IV	V	r	$\bar{y}$	Slope X on Y	Intercep
	milligrams LEAD/gram FEED								
	2	16	57	155	548				
Bone μg/gm	4.88	6.19	6.36	10.38	17.35	0.513	8.512	0.021	5.820
Blood μg/10 ml	0.86	1.47	2.47	3.23	7.09	0.841	2.612	0.006	1.596
Kidney μg/gm	0.22	0.41	0.74	1.17	2.91	0.923	0.954	0.005	0.355
Liver μg/gm	0.30	0.67	3.74	2.18	7.90	0.891	2.13	0.013	0.458
Brain μg/gm	0.05	0.07	0.10	0.12	0.55	0.869	0.149	0.001	0.034
Muscle μg/gm	0.06	0.06	0.04	0.04	0.10				

TABLE 8
SIGNIFICANT RESPONSES IN RATS AND DOGS FED
LEAD ACETATE FOR TWO YEARS

	Rats	Dogs
Dose, Pb in Feed, μg/gm	154	128
Lead in Urine, μg/10 ml	1.23	1.36
Lead in Feces, μg/gm	381	342
Lead in Urine, % dose fed	0.2	1.0
delta-Aminolevulinic Acid, μg/ml*	4.3	2.9
Stippled Cells/50 mf	0.54	0.05
Lead in Tissues		
Bone, μg/gm	16.70	8.51
Kidney, μg/gm	4.64	0.95
Brain, μg/gm	0.26	0.15
Blood, μg/10 ml	0.75	2.61
Liver, μg/gm	0.63	2.13

*Calculated from μg/day and 24 hour urine volume

FIGURE 4

Increase in the level of lead in dog tissue with increasing levels of lead in the diet