Chronic Plumbism in Rabbits: A Comparison of Three Diagnostic Tests

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SUMMARY

Three groups of rabbits (A, B, and C; 6 rabbits/group) were fed a lead supplement of 25, 50, and 100 mg of Pb/kg of live weight/day for 87 days to compare the efficacies of 3 diagnostic tests—whole blood lead concentration, urinary δ-aminolevulinic acid (UALA), and fluorescent erythrocyte test (FET)—and to determine the clinicopathologic changes of experimentally induced lead poisoning in rabbits.

All rabbits given lead had whole-blood lead concentrations greater than the maximum value (0.030 mg/dl) for control rabbits (group D), indicating that this measurement is a reliable indicator of lead ingestion.

All group A rabbits (fed 25 mg of Pb/kg) and 66% of the group B rabbits (fed 50 mg of Pb/kg) had false-negative UALA test results, with values less than the maximum value (0.12 mg/dl) for group D (control) rabbits. Only group C rabbits (fed 100 mg of Pb/kg) had consistently positive UALA findings. The test was therefore considered unreliable for detecting daily lead intakes less than 100 mg/kg of live weight of rabbits.

All rabbits given lead had erythrocytes which fluoresced red when exposed to light rays with wavelengths from 320 to 400 nm; fluorescence was not observed in erythrocytes of control rabbits. The FET appears to be a convenient and reliable diagnostic test for lead ingestion.

In groups B and C, clinical signs of lead poisoning were mild, nonpersistent anemia characterized by the presence of poikilocytes, hypochromic erythrocytes, target cells, erythroblasts, erythrocytes with punctate basophilic stippling, reduced mean corpuscular hemoglobin concentrations, and relative lymphocytosis, neutropenia, and eosinopenia. One rabbit from the group fed the largest dose displayed partial anorexia.

Few comparative studies have been reported on sensitivities of diagnostic tests for chronic lead ingestion. One test frequently used in examination for lead intoxication is the measurement of lead in whole blood.[5,20,25]

Another frequently used test, often used to screen urban children, is based on the inhibition of δ-aminolevulinic acid dehydrogenase by lead.[1] This inhibition results in the accumulation of UALA in lead-intoxicated rabbits, cows, dogs, cats, and man.[11,19,25]

Inhibition by lead of heme synthetase, another enzyme in the heme biosynthetic pathway, results in accumulation of protoporphyrin IX in the erythrocytes of lead-intoxicated rabbits and man. The protoporphyrin chelated with zinc is responsible for the red fluorescence of these erythrocytes when exposed to rays with wavelengths from 320 to 400 nm.[3,9,17] Fluorescing erythrocytes had been demonstrated in lead-intoxicated persons, mallard ducks *(Anas platyrhynchos)*, and Canada geese *(Branta canadensis)*.[4,26]

Assays for blood lead and UALA in man with lead poisoning have been compared in several reports.[6,16,30] Since the regimen and the quantity of lead intake was unknown in these studies, blood-lead values were used as standards against which UALA values were compared. Sometimes a subject had an increased blood lead value and a normal UALA concentration. In one study, frequency of false-negative test results was as high as 78%.[6]

An experimental chronic intoxication of rabbits was conducted to determine the sensitivities of several diagnostic tests for chronic lead poisoning and to study the relationship between the dosage of lead and the hematologic changes.

Materials and Methods

Twenty-four 4-week-old male New Zealand rabbits were fed a pelleted basal ration (Table 1). The rabbits were placed in individual metabolism cages in 4 groups (A, B, C, and D) of 6 rabbits each, and each group was given lead acetate supplement in its feed for 87 days as follows: Group A—25 mg of Pb/kg of body weight, group B—50 mg of Pb/kg, group C—100 mg of Pb/kg, and group D (controls)—no supplement.

Heparinized blood samples (10 ml) and 24-hour urine samples were collected from each rabbit on the 16th, 37th, and 84th day of the lead-feeding period. Erythrocyte and leukocyte counts, packed-cell volume, and hemoglobin determinations were made as soon as samples were collected. Blood smears were air-dried and stained with Wright's stain (without prior methanol fixation). Differential and stippled cell counts were made. All rabbits were killed on the 87th day of the lead-feeding period. Bone marrow smears from the femur were stained with Wright's stain and examined.

Blood-lead measurements, using the Delves sampling cup technique,[5] and FEP, were performed on blood samples taken on the 84th day.

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TABLE 1—Pelleted Basal Ration for Rabbits*

Ingredients**	kg/100 kg
Barley, grain, all analyses US (4) †‡ (ground barley)	30.8
Oats, grain, all analyses US (4) ‡ (ground oats)	22.5
Flax, seed, solvent-extracted, ground (52) (LSM solvent process)	8.7
Soybean, seed, without hulls, solvent-extracted, ground, maximum 3% fiber (53) (SOM 50%, solvent process)	8.7
Beet, sugar, pulp, extracted residue, dehydrated (10) ‡ (dried beet pulp)	10
Wheat, bran, dry-milled (4) (wheat bran)	22.5
Cane, sugar, molasses, mechanically expressed, minimum 48% invert sugar (4) (cane molasses)	9
Limestone, ground, minimum 32% calcium (80) (calcium carbonate)	0.750
Animal, bone, steamed dehydrated ground (80) (steamed bone meal)	0.350
Iodized salt	0.9
Yeast, irradiated, dehydrated ‖ (52)	0.005
Vitamin E supplement‖	0.55
Vitamin A supplement‡‡	0.0015

LSM = Linseed oil meal; SOM = soybean oil meal.
* From the Department of Nutritional Sciences, University of Connecticut, Storrs, CT. ** Nomenclature from National Academy of Science, National Research Council, Publication 1232, 1964. † Numbers in parentheses indicate the nutrient code (i.e., energy feed, protein, etc) as used in NAS-NRC food tables. ‡ Ingredients finely ground. ‖ Standard Brands (type 36-F, 36,000 IU of vitamin D/g contributes 2,360 IU of vitamin D/kg of ration. ‖ Hoffmann-LaRoche vitamin E acetate beadlets, 500 IU of *d, l*α-tocopheol acetate/g contributes 250 IU of vitamin E/kg of ration. ‡‡ Hoffmann-LaRoche type 325-40 vitamin A acetate beadlets, 325,000 USP units/g, contributes 1,500 μg of retinol equivalent/kg of ration.

A fluorescence microscope* equipped with a HBO 200 with mercury lamp and BG-38, BG-12, and BG-3 exciter filters were used to perform the FET. These filters provided an exciting light with a wide high peak from about 320 to 400 nm. Barrier filters 65, 50, and 44 resulted in a total pass band from 500 to 650 nm, with a wide high peak from about 550 to 650 nm. A drop of heparinized blood was placed on a glass slide with a coverslip gently pressed on top. The room was made completely dark. White light was used to focus before changeover was made to ultraviolet. The preceding technique varied from that described by Whitaker and Vietti[16] and by Barrett and Karstad[1] in that the barrier filters used in the present study screened out weak red fluorescence.

The 24-hour urine samples (10 ml) were treated as recommended by Ullman,[15] and UALA was measured according to the method described by Haeger.[7]

Results and Discussion

All rabbits consuming lead had increased blood lead concentrations greater than the maximum value from control (group D) rabbits (0.030 mg/dl) (Fig 1).

The groups of rabbits fed 25 and 50 mg of Pb/kg had similar mean whole blood lead concentrations (0.079 mg/dl), and the group fed 100 mg of Pb/kg had a mean whole blood level about 0.062 mg/dl higher (Table 2). It appears that a daily lead dose between 50 and 100 mg/kg exceeded the ability of the rabbits to clear the excess lead from the blood by urinary and biliary excretion or by deposition in bone and soft tissues.

None of the rabbits in group A (fed 25 mg of Pb/kg) and 2 of the 6 rabbits in group B (fed 50 mg of Pb/kg) had UALA concentrations exceeding the maximum value for controls (0.12 mg/dl) (Table 2, Fig 2), whereas all group C rabbits (fed 100 mg of Pb/kg) had

* Large Fluorescence Microscope (Universal), Carl Zeiss, Oberkochen/Württemberg, West Germany.

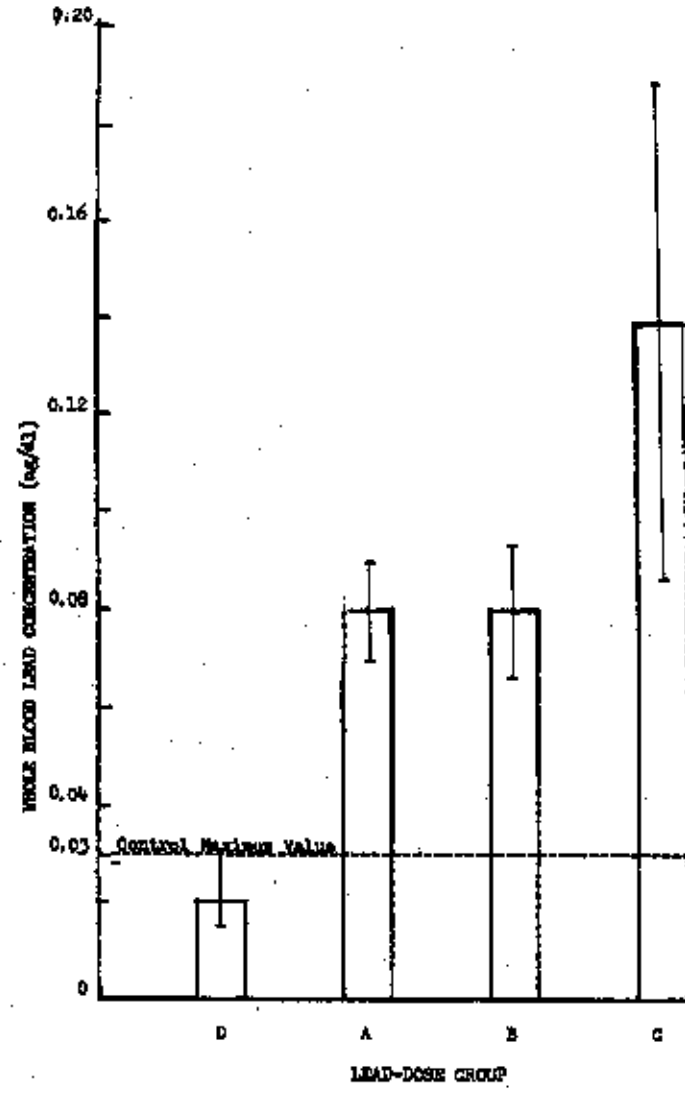

Fig 1—Mean whole blood lead concentration in group A, B, C, and D rabbits measured on the 84th day of the lead-feeding period. Vertical bars = minimal-maximal values.

TABLE 2—Mean Whole Blood Lead Concentrations, Urinary δ-Aminolevulinic Acid (UALA) Concentrations, and Fluorescent Erythrocyte Test (FET) Results for Rabbits Fed a Lead Supplement (84th Day of the Lead-Feeding Period)

Rabbit group (dose of Pb/kg/day)	Whole blood lead (mg/dl)	UALA (mg/dl)	FET
A (25 mg)	0.0795 (0.069–0.090)*	0.066 (0.06–0.08)	Positive
B (50 mg)	0.0793 (0.067–0.091)	0.100 (0.00–0.18)	Positive
C (100 mg)	0.1405 (0.011–0.150)	0.725 (0.42–1.48)	Positive
D 0 (Control)	0.0213 (0.017–0.030)	0.060 (0.00–0.12)	Negative

* Minimal-maximal values are shown in parentheses.

UALA concentrations above the maximum for control rabbits. A graph of 3 UALA excretion values during the lead-feeding period showed an increase in UALA, with continued lead ingestion for group C rabbits only (Fig 3). A false-negative UALA test for lead ingestion is one

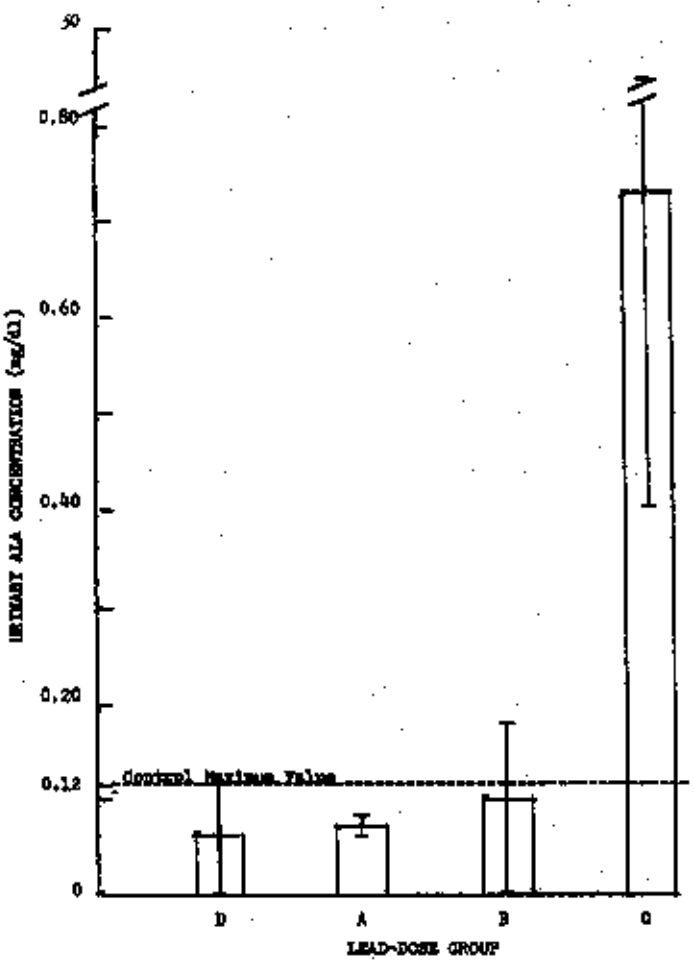

Fig 2—Mean δ-aminolevulinic acid (UALA) concentration in group A, B, C, and D rabbits, measured on the 84th day of the lead-feeding period. Vertical bars = minimal-maximal values.

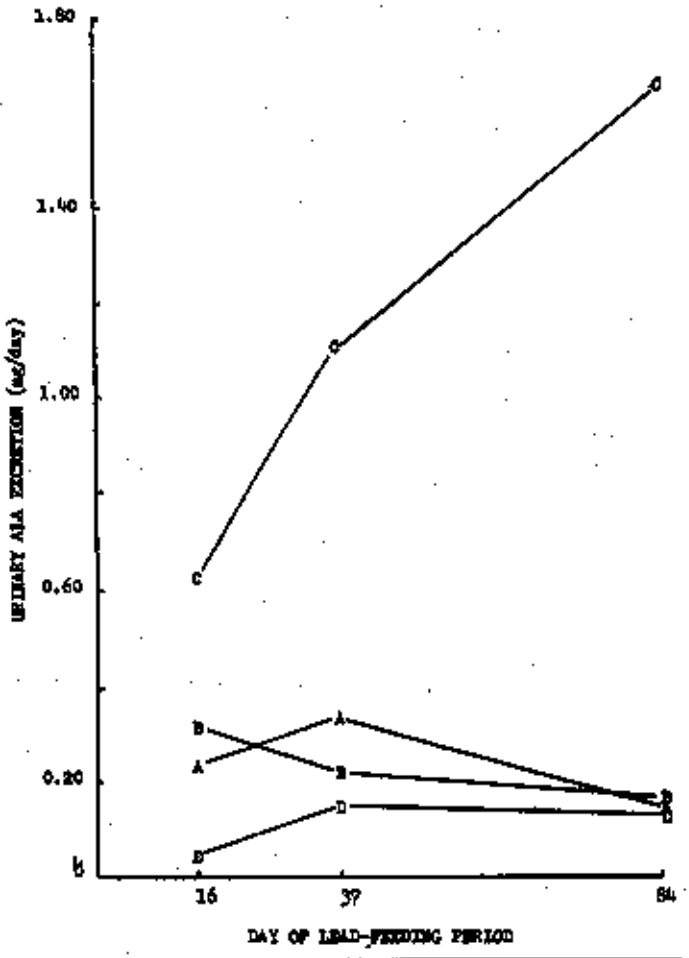

Fig 3—Effect of lead ingestion on UALA excretion by rabbits fed lead on 3 test days. A, B, C, and D indicate mean values for their respective groups.

in which UALA levels are at or below the maximum value for controls in spite of chronic lead ingestion. In our study, 100% false-negative tests for group A and 66% false-negative tests for group B rabbits indicated that the measurement of UALA concentrations is an unreliable test for chronic lead ingestion in rabbits given daily doses of 50 mg or less of Pb/kg of body weight.

All of the lead-fed rabbits had positive FET, whereas none of the group D (control) rabbits had positive tests (Table 2). The FET was reliable for rabbits given daily lead doses of 25 mg or more/kg. It should be noted that slow scanning of the wet blood smear was necessary to detect the short-lived red fluorescence. Strong positive tests showed individual fluorescent erythrocytes (fluorocytes), whereas in weaker positive smears there was a diffuse reddish hue to the field with no obvious individual fluorocytes. This hue was more noticeable at the thicker margins of the smear. In negative smears, the microscopic field was black, and red fluorescence was not detected. This test was still reliable when performed on blood which had been refrigerated for 1 month.

Erythrocyte fluorescence, as a diagnostic aid for lead poisoning, has not been widely used since the early work of Whitaker and Vietti in 1959.[15] This may have been due to the subjective nature of the test, which required an experienced technician to estimate the percentage of fluorescent erythrocytes in the microscopic field. This was necessary to distinguish lead-induced erythrocyte fluorescence and that associated with pernicious anemia, hepatitis, hemolytic anemia, and other less common conditions. A recent attempt to quantitate the FET, using a fluorometer to measure the intensity of the fluorescence, has shown promise as a mass screening tool for children.[16] This technique improves the specificity of the test by precisely defining the maximums of the exciting light (424 nm) and the emitted light (594 nm).[17] Another advantage of the fluorometric method is that it eliminates the need for an experienced technician.

The first clinical sign of lead intoxication was partial anorexia noticed in 1 of the group C rabbits (100 mg of Pb/kg), a finding consistent with clinical observations of lead-poisoned dogs.[7] Rabbits in groups A, B, and D were asymptomatic.

The low hemoglobin value (10 mg or less/dl) in 2 of the group B rabbits and 5 of the group C rabbits and the reduced mean corpuscular hemoglobin concentration (29 mg or less/dl) in 5 of the group C rabbits were manifested by the occurrence of target cells and

ypochromic erythrocytes in the peripheral blood. Although hemoglobin concentrations were not noticeably reduced in group A rabbits on the 84th day of the lead-feeding period, heme synthesis must have been affected, judging from the positive FEP results for these animals.

Studies of erythrocytic survival times in persons who have had lead intoxication indicate that lead shortens the life-span of most circulating erythrocytes.[15] This short erythrocytic survival probably explains the low erythrocyte counts (less than 3.86 million/mm^3) in group C rabbits (100 mg of Pb/kg), but the anemia was neither progressive nor persistent. Some of the anemic rabbits had a compensatory erythroid hyperplasia of the bone marrow which resulted in erythroblastemia (Fig 4). An increased erythropoietic activity has been seen in lead-intoxicated rabbits.[11]

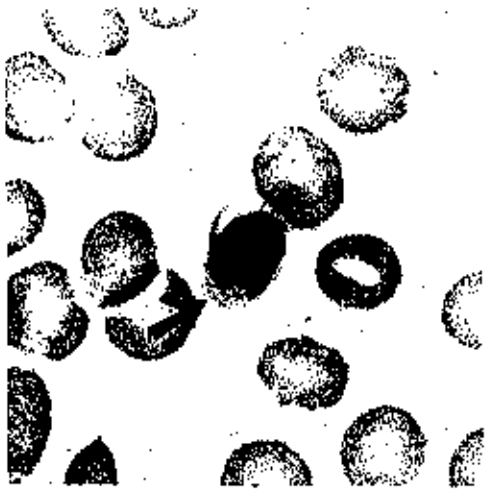

Fig 4—Blood smear of a group C rabbit (fed 100 mg of Pb/kg/day) on the 84th day of the lead-feeding period. Notice erythroblast (arrow). Wright's stain; × 1,000.

One of the most distinctive hematologic features of plumbism is the punctate basophilic stippling of erythrocytes, which has been reported for man, dogs, sheep, ducks, swine, gorillas, rats, and baboons.[1,2,10,11,18,22,24,26-28] Stippling is not pathognomonic for lead poisoning, since it has been observed in persons with thalassemia, hemolytic anemia, leukemia, and reticulum sarcoma and after exposure to benzene, aniline, carbon monoxide, arsenic, copper, and bismuth.[1,11,26]

The daily oral dose of lead that results in punctate basophilic stippling in peripheral blood of rabbits is probably at or near 25 mg of Pb/kg (Table 3) since

TABLE 3—Stippled Erythrocyte Counts of Peripheral Blood Smears from Rabbits Fed a Lead Supplement (84th Day of Lead-Feeding Period)

Rabbit group (dose of Pb/kg/day)	Stippled erythrocytes/1,000		
	16th	57th	84th
A (25 mg)	.....	.....	2 (2)
B (50 mg)	1 (1)	11 (2)	17 (4)
C (100 mg)	14 (5)	14 (5)	19 (3)
D 0 (control)	.....	.....	.....
Total	15 (6)	25 (7)	38 (9)

Data in parentheses indicate No. of rabbits.

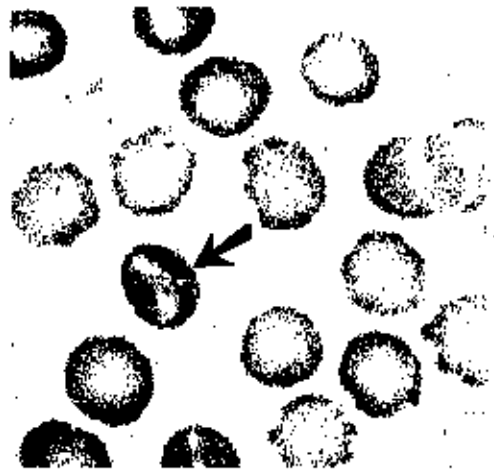

Fig 5—Blood smear of a group B rabbit (fed 50 mg of Pb/kg/day) on the 84th day of the lead-feeding period. Notice punctate basophilic stippled erythrocyte (arrow). Wright's stain; × 1,000.

erythrocytes with punctate basophilic stippling were observed only rarely (1 in 1,000 erythrocytes) in peripheral blood (Fig 5) of only 2 of the rabbits given 25 mg of Pb/kg and then only on the last blood sample-collecting day. Stippled cells were observed in blood smears in 5 group B rabbits and in 6 group C rabbits on at least 1 sampling day in the lead-feeding period. The total number of stippled erythrocytes and the number of rabbits affected tended to increase with continued lead ingestion for group A and B rabbits. However, the number of group C rabbits (fed 100 mg of Pb/kg) in which stippled erythrocytes were observed decreased from 5 on day 16 to 3 on day 84 of the lead-feeding period. Stippling was not persistent. Since feeding, blood sampling, and staining procedures were done in a uniform manner throughout the study, the disappearance and reappearance of stippled cells may be related to reticuloendothelial activity, as was suggested by Hopkins.[14] In any event, the absence of basophilic stippled erythrocytes in peripheral blood does not preclude a diagnosis of lead poisoning in rabbits.

Examination of bone marrow smears failed to reveal punctate basophilic stippled erythrocytes. Since the marrow smears were made 3 days after the last day of blood sampling and since peripheral blood smears were not examined, perhaps stippled cells were not being produced at the time of marrow sampling. Alternatively, it is possible that basophilic stippling does not occur in erythroblasts of New Zealand White rabbits.

Anisocytosis and poikilocytosis were pronounced in the group C rabbits (fed 100 mg of Pb/kg), although all rabbits, including controls, had some degree of anisocytosis and poikilocytosis. Schermer[21] describes rabbit erythrocytes as being highly anisocytotic and frequently microcytotic. "Thornapple" forms of erythrocytes are also a common finding in normal rabbit blood smears.[21]

Lead-dosed rabbits often displayed relative lymphocytosis (80% or greater) and neutropenia (9% or less) but no eosinopenia. These observations were made mainly on the group C rabbits. Relative lymphocytosis has been recorded[12,16] for rabbits and white hamsters experimentally intoxicated with lead.

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