PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 663–668 (1974)

Time Course of Hematologic Changes During Chronic Lead Poisoning[1] (37871)

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Experiments with chronically lead-poisoned animals have shown relatively poor agreement in the observed changes of oxidative and enzymatic activity of circulating red cells (1–4). Erythrocytes from lead-poisoned individuals, however, have been shown to be functionally and structurally defective as indicated by a shortened red-cell life span (5), increased mechanical fragility (6), and alteration in membrane permeability (7). These erythrocyte defects have been attributed to the interaction of lead with the red cell membrane (8), but there is good evidence that lead also acts at intracellular sites (9, 10). For example, lead interferes with the ability of erythroblasts to synthesize heme, resulting in a decreased hemoglobin concentration and accumulation of delta-aminolevulinic acid (11). Waldron (12) has suggested that functional defects in erythrocytes of lead-poisoned individuals may be due to the effect of lead during the early stages of erythrocyte development. Thus, we might expect to find significant changes in the metabolism of erythrocyte precursors, such as reticulocytes, which during development in the bone marrow may be exposed to relatively high lead concentrations (13).

To test this hypothesis, we studied the hematologic changes in young, rapidly growing rats maintained on a diet containing 1% lead acetate. Primary interest was directed toward the reticulocyte because of its relative ease of isolation after release from the bone marrow. Catalase and adenosine triphosphatase (ATPase) activity, two enzymes important in the maintenance of the functional integrity of the red cell, were studied, and reticulocyte oxygen consumption was used as an index of cell oxidative metabolism during the chronic lead poisoning.

Materials and Methods. Twenty-one male Wistar rats (160 g) were fed granulated Purina Chow containing 1% lead acetate (PbAc) *ad lib.* for 24 weeks, and 21 controls received the same diet without PbAc. Based on their average food intake, each rat ingested about 125 mg PbAc/day or 68.3 mg Pb/day. Three animals from each group were exsanguinated after 3, 6, 9, 12, 15, 20, and 24 weeks of feeding. One week prior to exsanguination, the rats were weighed, and blood samples were obtained by tail venipuncture for standard hematologic measurements of hematocrit (Hct), hemoglobin (Hb), reticulocyte, and siderocyte counts (14). Blood lead was determined by a modification of the Delves microcup technique for AAS (15). Three rats from each group received three alternate-day ip injections of phenylhydrazine–HCl (4 mg/100 g) to induce a reticulocytosis (16). At the end of the phenylhydrazine treatment, the rats were decapitated, and their blood was collected through a heparinized nylon filter and pooled in vessels containing cold heparinized 145 m*M* NaCl solution.

The pooled blood (25 and 30 ml) from control and lead-fed rats, respectively, was

[1] This investigation was supported by a grant from the International Lead Zinc Research Organization and General Support Grant No. 5409, National Institutes of Health.

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washed 3 times at 750*g* for 10 min in a refrigerated centrifuge maintained at 4°. After centrifugation, the supernatant and buffy coat was discarded, and the packed red cells were resuspended (2:1 v/v) in a solution containing 145 m*M* NaCl, 6 m*M* KCl, and 50 m*M* glucose, and adjusted to pH 7.4 with Tris-Cl (Na–K–glucose). Total cell counts were done on each sample with a Model B Coulter Counter, and the percent reticulocytes was determined by counting New Methylene Blue-stained smears. A 0.2-ml aliquot of the reticulocyte-rich washed packed cells from each control and experimental sample was used to measure reticulocyte oxygen consumption as described below. The remainder of the packed cells were hemolyzed in 8 vol of 10 m*M* Tris-Cl (pH 7.4) for isolation of red-cell plasma membranes by the method of Dodge *et al.* (17). The final membrane pellet (18,400*g* for 20 min) consisted of a brownish bottom layer and a pink fluffy upper layer relatively free of hemoglobin. The small dark-red button beneath the brownish layer was discarded, and the membrane pellet was resuspended in 8 ml of Na–K–glucose solution and immediately assayed for catalase activity by the polarographic method of Goldstein (18). The linear initial rate of the oxygen curve was used to calculate catalase activity of the red cell membranes as μl oxygen released/mg protein/hr.

The remainder of the membrane suspension was frozen for 24 hr, then thawed, and the sodium plus potassium-dependent ATPase activity was measured by the method of Post *et al.* (19). After 60 min, the reaction was stopped by addition of 0.5 ml of 5% trichloroacetic acid, and the phosphate content of the supernatant was determined colorimetrically (20). ATPase activity was calculated as μmoles inorganic phosphate released/mg protein/hr, and corrections were made for endogenous inorganic phosphate. The protein content of membrane preparations was determined by the Lowry method (21).

The oxidative activity of 0.2 ml of reticulocyte-rich packed cells in 3.8 ml of Na–K–glucose solution was measured with a YSI Model 53 oxygen electrode system in stirred, air-equilibrated suspensions at 37°. Since erythrocytes under these conditions consume negligible quantities of oxygen, the red cell respiration was expressed as a function of the number of reticulocytes in the sample (μl oxygen/10^9 reticulocytes/hr). Tests for statistical significance were done by Student's *t* test for small sample size, using an Olivetti Underwood Programma 101 desk computer.

Results. The presence of 1% lead acetate in the diet of rapidly growing, young rats

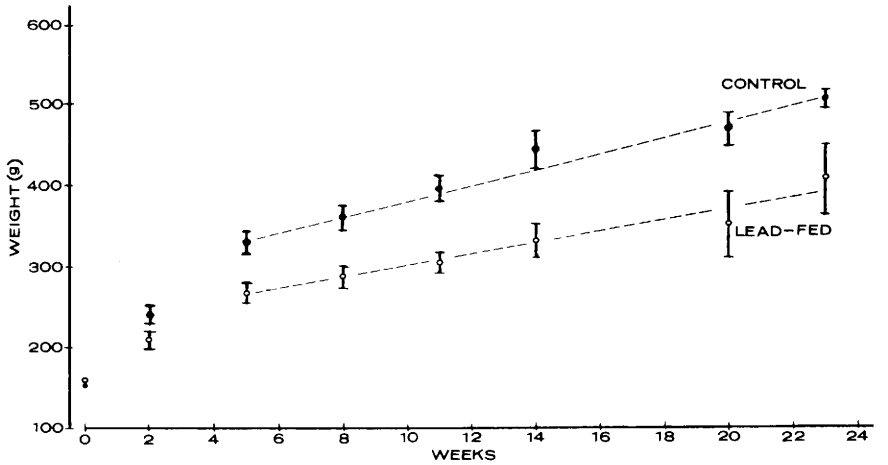

FIG. 1. Average weight of rats during period of lead intoxication. The bars represent the standard deviation, and the dotted lines indicate the regression line calculated from values obtained between the 5th through the 23rd weeks.

TABLE I. Blood Lead and Hematology of Control and Lead-Fed Rats.[a]

	Week							
	0	2	5	8	11	14	19	23
Blood lead (μg%)								
Control	17	20	22	24	25	22	24	25
Pb fed	18	85[c]	84[c]	112[c]	142[c]	153[c]	152[c]	154[c]
Hematocrit								
Control	42.9	44.6	50.0	48.8	50.1	50.7	49.0	49.0
Pb fed	43.4	44.8	48.0	49.6	45.6[c]	46.0[b]	48.8	48.8
Hemoglobin (g%)								
Control	14.0	14.2	14.4	15.3	15.3	15.8	16.3	16.6
Pb fed	14.3	14.9	15.0	14.9	14.0[c]	14.4[b]	15.2[b]	13.8[c]
Mean corpuscular hemoglobin conc.								
Control	32.6	31.9	28.8	31.4	30.5	32.0	33.3	33.7
Pb fed	32.9	33.4	29.4	30.3	30.8	31.3	31.8[c]	28.1[c]
Reticulocytes (%)								
Control	2.1	2.0	2.2	1.4	1.8	1.6	1.4	1.8
Pb fed	1.9	4.9[c]	2.2	3.1[b]	3.5[b]	2.8[b]	3.1[c]	2.2

[a] Data is expressed as the mean of five determinations.
[b] Significantly different from control, $P < .05$.
[c] Significantly different from control, $P < .01$–$.001$.

slowed their rate of growth 20% as compared to the controls (Fig. 1). Blood lead remained below 25 μg/100 ml in the controls, but in the lead-fed rats rose to approximately 140 μg/100 ml in 2 days and then, after some fluctuation, dropped to an average of 85 μg/100 ml for the first 5 weeks (Table I). During the latter part of the experiment, blood lead rose above 100 μg and averaged 152 μg/100 ml during weeks 14–23. In spite of the high blood lead levels of the lead-fed rats, hematologic signs of the lead poisoning were sparse during the first 8 weeks. Hemoglobin, hematocrit, and mean corpuscular hemoglobin remained at about control levels, and elevated reticulocyte counts were observed only on the 2nd and 8th weeks (Table I). By the 11th week, the lead-fed rats showed definite signs of anemia as indicated by a reticulocytosis coupled with a significant decrease in both hematocrit and hemoglobin concentration. Although blood lead varied from week to week, it remained consistently high, but hematocrit values unexpectedly returned to approximately control levels by the 19th and 23rd weeks while hemoglobin concentration remained depressed. Siderocytes were not detected in the blood of either control or lead-fed animals during the course of the experiment.

The mean oxygen uptake of reticulocytes obtained from phenylhydrazine injected control rats ranged from 21.1 to 24.3 μl oxygen/10^9 reticulocytes/hr during the experimental period (Table II). Reticulocyte oxygen uptake of lead-fed animals showed no significant changes from control values during the initial 9 weeks of lead exposure. When measured on the 12th week, the oxidative activity of reticulocytes from the lead-fed rats fell below control values, and in subsequent measurements on the 15th through the 24th weeks, reticulocyte oxidative activity of the lead-intoxicated rats remained significantly below control levels.

The blood samples, obtained from phenylhydrazine-treated animals, consisted of mixtures of erythrocytes and reticulocytes. Since immature red cells (reticulocytes) have a much higher metabolic activity than erythrocytes, variations in control ATPase activity were attributed to the percentage of reticulocytes in the packed cell samples from which the membranes were isolated, and ATPase activity was recalculated to correspond to

TABLE II. Reticulocyte Oxygen Consumption and Enzymatic Activity of Control and Lead-Fed Rats.[a]

	Week						
	3	6	9	12	15	20	24
0_2 uptake (μl 0_2/10^9 reticulocytes/hr)							
Control	22.7	23.4	21.1	24.8	22.5	22.2	24.3
Pb fed	21.5	20.8	20.4	19.8[c]	19.2[c]	18.7[c]	19.5[c]
	(4)[d]	(3)	(3)	(4)	(4)	(4)	(4)
ATPase (P_i/mg protein/hr)							
Control	1.85	1.61	1.68	2.10	2.04	3.71	3.59
Pb fed	1.34[c]	1.40[c]	1.40[c]	1.79[c]	0.83[c]	1.54[c]	1.78[c]
	(7)	(7)	(8)	(6)	(7)	(10)	(9)
Catalase (μl 0_2/mg protein/hr)							
Control	305	311	296	294	309	299	307
Pb fed	314	348[b]	325[b]	339[b]	283[c]	267[c]	251[c]
	(3)	(5)	(6)	(7)	(5)	(5)	(6)

[a] Data is expressed as the mean of the number of determinations in parentheses.
[b] Significantly different from control, $P < .05$.
[c] Significantly different from control, $P < .01$–$.001$.
[d] Number of determinations.

the ATPase activity of samples with 50% reticulocytes (Table II). Control ATPase activity (adjusted to 50% reticulocytes) ranged from 1.6 to 3.7 μmoles P_i/mg protein/hr during the 24-week experimental period, but red-cell membranes from the lead-fed rats were significantly below control values during the entire experimental period. In the lead-fed rats, the ATPase depression preceded the observed decrease in reticulocyte oxidative activity by several weeks.

Catalase activity of control red-cell membranes showed little variation with respect to the percent reticulocytes. Red-cell membranes isolated from control rats had catalase values ranging from 294 to 311 μl O_2/mg protein/hr (Table II). Catalase activity was slightly elevated in animals fed lead for 3 weeks, but during weeks 6, 9, and 12, the lead-fed group showed a significant increase in catalase activity. The catalase activity of red-cell membranes obtained from lead-intoxicated rats fell below control values on the 15th week and remained significantly lower than the controls until the termination of the experiment.

Discussion. Our enzyme studies are of a preliminary nature, but they do indicate that significant changes in ATPase and catalase activity occur in the cells of lead-intoxicated animals. Apparently, the activity of these two enzymes changes fairly rapidly during the early period of chronic lead exposure. Altered ATPase activity undoubtedly causes shifts in the sodium–potassium exchange which would disturb the osmotic balance of red cells exposed to lead (4). The ability of catalase to destroy peroxide in red cells is related to an increased rate of hemolysis, and decreased life span in catalase-deficient cells (22). Although we observed increased catalase activity during the early phases of the chronic lead poisoning, there was a significant fall in activity by the 15th week. A severe decrease in membrane catalase activity in plumbism could lead to a shortened life span of the circulating red cells. There have been previous reports of increased erythrocyte catalase activity during the early stages of experimental plumbism (23, 24) which showed signs of diminution after about 9 weeks. Minden *et al.* (25) and Haeger-Aronsen (26) also reported that liver catalase activity was depressed in lead-poisoned animals.

It is difficult to explain the fluctuation in blood-lead level during chronic lead poisoning of rats kept on a diet containing 1% PbAc (Table I). We have, however, seen similar fluctuations in a number of experiments and suggest that variations in blood

lead may represent patterns of lead mobilization and excretion in these growing animals. The periodic hematologic symptoms of lead poisoning we observed may be explained by oscillations of mobile lead in the bone marrow (13). Cardona and coworkers (27) showed that the inhibitory effects of lead on mitochondrial oxidative phosphorylation can be reversed by increased levels of exogenous inorganic phosphate. A similar condition may prevail in the bone marrow where the extracellular and intracellular phosphate content may be sufficient at times to prevent the toxic effects of lead on erythrocyte precursors. It may be that as lead accumulates in the bone marrow, the protective action of inorganic phosphate and other lead-inhibiting substances is overcome and significant damage occurs to the developing red cells. This proposed explanation is consistent with clinical findings in individuals chronically exposed to lead who from time to time show a moderate or severe anemia, and at other times are not anemic at all (28, 29).

Our studies on the respiration of circulating reticulocytes in chronic lead intoxication contrasts with the findings of some earlier investigators. For example, Hernberg and coworkers (1) found no significant changes in the oxygen consumption of peripheral red cells obtained from industrial workers exposed to lead, but Baikie and Valtis (2) noted an increased oxygen uptake in blood samples obtained from lead-poisoned rabbits. Since the metabolism of mature erythrocytes is essentially glycolytic (30), a change in the oxygen utilization of peripheral red cells in lead poisoning should not occur until there has been a substantial release of juvenile cells into the peripheral circulation (as noted by Baikie and Valtis). Although the effect of lead on the pentose shunt cannot be excluded, the oxidative inhibition we observed with reticulocytes obtained from lead-fed rats suggests a significant effect of lead on reticulocyte mitochondrial activity. It is not unlikely that a similar depression in oxygen utilization would be observed with other cell types of the erythropoietic series studied under similar conditions. There is evidence of structurally defective erythroblast mitochondria in cells obtained from lead-poisoned rats (31), as well as evidence for the inhibition of hemoglobin synthesis by lead, which suggest functional disturbances in erythrocyte precursor mitochondria. Malfunctions of metabolic activity, induced by lead early in erythropoiesis, have a deleterious effect on red-cell development and may impair the ability of erythrocytes to survive for a normal period in the circulation. Our results on the effect of lead on reticulocyte metabolism support the hypothesis that the anomalous function of red cells in lead poisoning may be traced to the action of lead on erythrocyte precursors.

Summary. Lead acetate (1%) added to the diet of young, rapidly growing rats slowed their growth 20%. Blood lead averaged about 90 μg/100 ml for the first 8 weeks and then rose to 150 μg during weeks 11–23. In spite of the high blood-lead levels, hematologic signs of lead poisoning were sparse until the 8th and subsequent weeks. Reticulocytes isolated from the lead-fed rats had lowered oxidative activity for the first 9 weeks, and were significantly depressed below controls in the 12th–24th weeks. The ATPase activity of cell membranes isolated from reticulocytes of lead-fed rats was significantly depressed during the entire study, but catalase activity initially rose and then fell during the latter part of the experimental period. The metabolic disturbances observed in these reticulocytes reflect the deleterious effect of lead on red-cell precursors and red-cell development.

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Received Sept. 4, 1973. P.S.E.B.M., 1974, Vol. 145.