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The Effect of Chronic, Low-Level Lead Poisoning on the Erythropoietin Response to Hypoxia[1] (40151)

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Anemia is a characteristic of chronic lead-poisoning (1). Although lead might cause anemia solely via its known interference with hemoglobin synthesis (1), lead might also affect the kidney's secretion of erythropoietin (ESF) since lead accumulates in the kidney (2) and is known to impair other renal functions (3). Moreover, with regard to the kidney's endocrine function, we have recently demonstrated that small, acute, iv doses of lead (0.1 mg/kg) raise plasma renin activity (PRA); in contrast, chronic lead poisoning in humans on low salt diets is associated with relatively low PRA (4, 5). There have been no studies of ESF in chronic lead poisoning, but in rats, extremely large, acute, iv doses of lead (40 mg/kg) either reduce (6) or have no effect on (7) the plasma ESF response to hypoxia on the subsequent day. We therefore hypothesized that chronic, low-level, lead-poisoning would reduce the ESF response normally seen in hypoxia; we have tested this hypothesis in rats.

Methods. Experimental protocol. Studies were performed on 48 male Sprague–Dawley rats weighing 355–469 g at the start of the study. For a 6-week period, four groups of 12 rats ate regular rat chow (Tecklad) and drank distilled water containing no lead (Group C—control or non-lead), 0.2 mg Pb/ml as lead acetate (group L,—low dose), 0.4 mg Pb/ml (group M-Medium dose), or 0.6 mg Pb/ml (group H—High dose). Rats were housed four to a cage, and daily fluid intake in each group-cage was measured throughout the six weeks. Thereafter, rats were transferred at 1400 hr to one of two pressure chambers, each of which could accommodate eight rats; food and water were available. Two rats from each of the 4 groups were placed in each chamber; for the next 22 hr pressure was left at atmospheric in one chamber (nonhypoxic) and was lowered to 0.5 atm in the second chamber (hypoxic). Flow rates of air through each chamber were great enough so that the gas composition in the chambers was the same as room air. Other environmental factors such as pumping noises were identical in both chambers. At 1200 hr the next day, rats were removed from the chambers, anesthetized immediately with sodium pentobarbital (27.5 mg/rat ip) and blood samples obtained via aortic puncture. All blood samples were obtained within 15 min after removal from the chamber, and the order in which rats from various groups were bled over the 15 min was randomized. Variations in erythropoietin due to variable sampling time were negligible since the half-time for ESF disappearance in the rat is on the order of 1–5 hr (8).

Analytical methods. Erythropoietin was estimated indirectly as previously described (9) and is reported as the % ^{59}Fe uptake by polycythemic mice (9) injected ip with rat-plasma over a 2-day period. The total volume of plasma injected was 3.25% of the mouse's weight (1.75% the first day, and 1.5% the second day; these dosages were chosen to utilize fully the amount of rat plasma that was available). Plasma from each rat was tested on three to five different mice, and the uptakes of these different mice were averaged (for each rat) prior to statistical analysis. Given that the erythropoietin assay depends on ^{59}Fe uptake, it is crucial that the lead transferred to the mice via the rat plasma not directly inhibit ^{59}Fe uptake (by inhibiting heme synthesis, a known effect of lead). We did not directly measure lead concentrations in the rat plasma, but our data on erythrocyte and total blood lead (see Table I) indicate that only 1–2% of the total blood lead was in the plasma; this is consistent with data of others (1) documenting that plasma lead is usually less than 10% of total blood lead. Accordingly, even taking a figure of 10%, the

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TABLE I. EFFECT OF HYPOXIA ON CONTROL AND CHRONICALLY LEAD-POISONED ANIMALS.[a]

	Non-hypoxic animals				Hypoxic animals			
	Control	low dose	med dose	high dose	Control	low dose	med dose	high dose
1. Blood lead (μg/100 ml)	<5	17.8 ± 2.3**	27.6 ± 7.2**	28.5 ± 2.5**	<5	27.0 ± 1.9**	44.4 ± 2.3**	46.1 ± 2.9**
2. Red Cell lead μg/g wet weight	<0.1	0.39 ± 0.05**	0.63 ± 0.17**	0.65 ± 0.05**	<0.1	0.55 ± 0.04**	0.94 ± 0.07**	0.93 ± 0.05**
3. Kidney lead μg/g of wet weight	<0.3	6.4 ± 0.8**	10.7 ± 1.2**	13.7 ± 0.9**	<0.3	7.6 ± 0.8**	13.6 ± 1.8**	15.0 ± 1.2**
4. Kidney lead (μg/kidney)	<0.5	11.5 ± 1.3**	19.5 ± 7.6**	27.1 ± 2.1**	<0.5	12.7 ± 1.2**	20.7 ± 2.7**	25.9 ± 1.6**
5. Bone lead femur shaft (μg/g wet weight)	<0.5	19.9 ± 2.4**	40.2 ± 5.8**	47.7 ± 2.4**	<0.5	21.3 ± 1.1**	36.6 ± 2.5**	41.1 ± 2.9**
6. Kidney Weight (2 kidneys)	3.43 ± 0.19	3.58 ± 0.18	3.62 ± 0.08	3.98 ± 0.09*	3.03 ± 0.10	3.35 ± 0.09*	3.04 ± 0.05	3.51 ± 0.12*
7. Hematocrit (%)	46.1 ± 0.6	45.5 ± 0.8	44.4 ± 0.5*	43.5 ± 1.1	47.7 ± 1.1	49.5 ± 0.5	47.6 ± 1.0	49.3 ± 0.6
8. Spleen Weight	0.833 ± 0.048	0.910 ± 0.053	0.955 ± 0.023	0.963 ± 0.052	0.614 ± 0.020	0.679 ± 0.037	0.642 ± 0.047	0.672 ± 0.029

[a] All values are mean ± SE. P values indicated with asterisks (* = $P < 0.05$, ** = $P < 0.01$) indicate significant differences between the lead treated and control groups under the same atmospheric pressure condition. When lead levels in tissues of control-animals were undetectable, the variability of the extraction blank (see ref 10) was used for statistical purposes to calculate the P values comparing lead treatment to the control group.

maximal plasma concentration in our rats receiving the highest lead dose can be assumed to be no greater than 2.8 μg/100 ml; therefore, the lead transferred was no greater than 0.025 μg. If this lead had all remained in the blood, mouse blood concentration would have been increased no more than 0.8 μg/100 ml; this is at least 20- to 30-fold lower than the amount required to alter heme synthesis directly.

Lead was measured by atomic absorption as described elsewhere (10). Hematocrit was determined on a microhematocrit reader after centrifugation on an International Microcapillary centrifuge. Student's *t* test was employed in the statistical analysis and all group values are expressed as mean ± SE.

Results and discussion. During the 6 weeks of chronic lead poisoning there was an inverse relationship between the volume of water drunk and the lead concentration in the water (C - 77.2 ± 1.60, L = 67.65 ± 1.44, M = 55.2 ± 1.00, and H = 56.91 ± 1.27 ml/day/rat); this corresponds to lead intakes of 13.5 (L), 22.1 (M) and 34.1 (H) mg/rat/day. Increased ingestion of lead did not affect body weight, but caused progressive increases in blood, kidney and bone lead (Table 1, lines 1–5), and produced signs consistent with (but not diagnostic of) mild lead-poisoning as previously observed in rats (2): i.e., increased kidney weight (line 6) and a tendency toward anemia (line 7).

Hypoxia caused several non-endocrine effects. Body weight fell from 471.9 ± 5.66 to 451.4 ± 4.64 ($P < 0.01$) after hypoxia (all dose-groups combined). The probable cause of this weight loss is a combination of decreased food intake (authors' personal observation) and increased respiratory and renal water loss (11). Spleen weight (Table I, line 8) fell during hypoxia ($P < 0.01$) in each of the 4 groups. Hematocrit rose during hypoxia in each lead-treated group ($P < 0.02$), but not in the control (non-lead) group (Table I, line 7); thus, lead poisoning appeared if anything to enhance the elevation in hematocrit during acute hypoxia. Since most of the hematocrit rise over the first 24 hr of hypoxia is due to reduction in plasma volume (12, 13), rather than formation of new red cells, it is likely that this reduction in plasma volume may have been greater in the lead-poisoned

animals. Hypoxia did not alter kidney or bone lead (Table I); but it did raise blood lead ($P < 0.02$, $P = 0.08$, $P < 0.001$ for groups L, M, and H respectively) partly as a result of the elevated hematocrits. However, hemoconcentration is not the sole mechanism, since hypoxia raises red-cell lead per se about 50% in the lead-treated groups ($P < 0.05$ for L, n.s. for M, $P < 0.01$ for H); we have no explanation for this finding.

The major finding in this study was that chronic lead-poisoning blunted the ESF response to hypoxia in a dose dependent manner (Fig. 1). EFS was undetectable in non-hypoxic rats; the iron uptakes after injection of plasma from these rats (Fig. 1) were not significantly different from uptakes in saline-injected mice (1.0 ± 0.6% Fe uptake). Thus, we can not conclude whether or not lead-poisoning per se, or its associated anemia, affects basal ESF activity. It is clear however, that any such effect of lead must be small compared to the effect of hypoxia itself. It has been previously shown that a large dose of lead (40 mg/kg) given 24 hr prior to hypoxia reduced the plasma ESF response to 2 hr hypoxia (6); however in a later study, the same researchers concluded that although the plasma ESF response to 5 hr hypoxia was normal in such lead treated animals, the lead-treatment shifted the source of much of the ESF from kidney to liver (7). Our results show that chronic lead treatment does impair the ESF response and although our studies do not permit us to localize the site at which chronic lead treatment interferes with ESF production, we believe the most likely site is the kidney, since, in the rat, it normally is the source of at least 90% of the ESF released in response to hypoxia (7).

The fact that lead poisoning blunted the ESF response to hypoxia is particularly significant, since the degree of lead poisoning in these experiments, as evaluated by tissue lead levels, was quite moderate. Blood lead levels 17.8 (L), 27.6 (M), and 28.5 (H) μg/100 ml in lead-treatment groups were comparable to those previous reported for wild rats living in rural (17 ± 0.02 μg/100 ml) and urban (55 ± 0.04 μg/100 ml) environments (10); moreover kidney lead in wild rats (1.14 ± .28 μg/g for rural and 22.7 ± 2.8 μg/g for urban, ref. 10) also covers the same range as achieved in

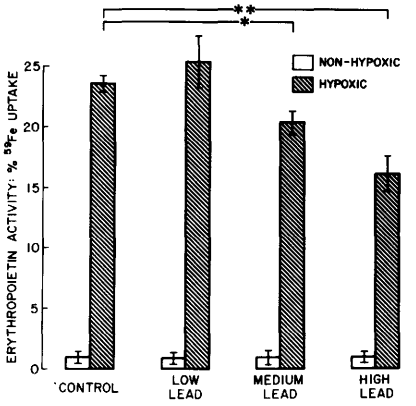

FIG. 1. Effect of hypoxia on plasma erythropoietin activity in control (non lead-treated) rats and rats that drank water containing lead concentrations of 0.2 mg/ml (low lead), 0.4 mg/ml (medium lead) and 0.6 mg/ml (high lead). In all four groups hypoxia caused a highly significant increase in erythropoietin activity ($P < 0.001$), but the response was significantly blunted in the two highest lead-treatment groups. ** = $P < 0.01$; * = $P < 0.05$. All values are mean ± SE.

these experiments (Table 1). Comparison of the blood lead levels achieved in the present experiments to those found in humans also suggests that the lead-poisoning was not extreme compared to that encountered in our environment; blood lead levels under 30 μg/100 ml in humans are not even considered indicative of lead poisoning (1). Thus, we conclude that the degree of lead poisoning in the treated rats was slight to moderate and that the findings could have significance for even slightly lead poisoned humans; failure of adequate endocrine control of erythropoiesis may thus contribute to the anemia of lead poisoning.

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