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S. R. V. Raghavan , B. Dwight Culver & Harvey C. Gonick

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ERYTHROCYTE LEAD-BINDING PROTEIN AFTER OCCUPATIONAL EXPOSURE. II. INFLUENCE ON LEAD INHIBITION OF MEMBRANE Na^+,K^+-ADENOSINETRIPHOSPHATASE

S. R. V. Raghavan

Department of Medicine and Trace Element Laboratory, Cedars-Sinai Medical Center, and University of California Center for Health Sciences, Los Angeles, California

B. Dwight Culver

Department of Community and Environmental Medicine, University of California, Irvine, California

Harvey C. Gonick

Department of Medicine and Trace Element Laboratory, Cedars-Sinai Medical Center, and University of California Center for Health Sciences, Los Angeles, California

Membrane Na^+,K^+-adenosinetriphosphatase in erythrocytes from three groups of industrially exposed Pb workers (without toxicity, with toxicity associated with high blood Pb levels, and with toxicity associated with low blood Pb levels) was inversely correlated with Pb in the membrane fraction but not significantly correlated with total erythrocyte Pb. This difference was attributable to the proportion of erythrocyte Pb bound to hemoglobin and a Pb-binding protein of molecular weight 10,000.

INTRODUCTION

We previously demonstrated that the Pb in erythrocytes from industrially exposed individuals is bound primarily to hemoglobin and to a newly discovered protein of molecular weight 10,000. This protein was not detectable in erythrocytes from normal individuals (Raghavan and Gonick, 1977). In a subsequent study (Raghavan et al., 1980), we also

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Requests for reprints should be sent to Harvey C. Gonick, Department of Medicine and Trace Element Laboratory, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, California 90048.

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found that the Pb bound to this protein was significantly decreased in a group of workers with evidence of clinical and biochemical toxicity at low total blood Pb levels (43–54 μg%). On the basis of these results, we suggested that workers who have a diminished capacity for synthesizing this low-molecular-weight Pb-binding protein may be at increased risk of developing Pb toxicity at relatively low blood Pb levels because of redistribution of Pb to "residual" erythrocyte fractions that contain critical Pb-sensitive enzymes (Raghavan et al., 1980).

It is now well documented that Pb has a strong affinity for -SH groups (Bonsignore et al., 1965; Hernberg and Nikkanen, 1972) and thus inhibits the sulfhydryl-containing enzyme δ-aminolevulinic acid dehydratase (ALAD), which is commonly used as a marker for Pb intoxication (Haeger-Aronsen et al., 1971). Na^+,K^+-activated adenosinetriphosphatase (Na^+,K^+-ATPase) is a sulfhydryl-containing membrane-bound enzyme that mediates active transport of electrolytes across biological membranes (Post et al., 1960). Prior studies demonstrated that red blood cell membrane Na^+,K^+-ATPase is also inhibited in Pb poisoning (Hasan et al., 1967b; Hernberg et al., 1967; Secchi et al., 1968). These studies, however, failed to demonstrate a significant inverse correlation between red blood cell membrane Na^+,K^+-ATPase and whole-blood Pb.

In the present investigation, we extended our observations concerning the distribution of Pb in various fractions of red blood cells from normal controls and Pb-exposed workers and, in particular, sought to determine whether there is a significant relation between Pb in the membrane fraction of the red blood cell and membrane Na^+,K^+-ATPase activity. As in our earlier study (Raghavan et al., 1980), we examined three groups of Pb workers (without toxicity, with toxicity associated with high blood Pb levels, and with toxicity associated with low blood Pb levels) to determine whether a deficiency in the low-molecular-weight Pb-binding protein might predispose to redistribution of Pb to the membrane fraction as well as to the prehemoglobin high-molecular-weight fraction of the red blood cell, where other Pb-sensitive enzymes are found.

METHOD

The study population consisted of 8 normal controls, 14 Pb workers without toxicity, 5 Pb workers with toxicity at high blood Pb levels, and 5 Pb workers with toxicity at low blood Pb levels. These groups were described in our earlier publication (Raghavan et al., 1980). Briefly, the normal controls were selected from a group of male industrial workers, 25–45 yr old, who had not been exposed to Pb. All three groups of Pb-exposed workers were selected from a single Pb smelting plant. Those with toxicity at high blood Pb levels had been referred for chelation treatment because of symptomatic and biochemical evidence of toxicity.

In all instances, total blood Pb levels exceeded 80 μg% and urine δ-aminolevulinic acid (ALA) and coproporphyrin levels were above normal. The Pb-exposed workers with toxicity at low blood Pb levels were identified because of their presentation at the plant with symptoms suggestive of toxicity, elevated urine ALA and, in some instances, urine coproporphyrin, but blood Pb levels consistently below 80 μg%. The Pb workers without toxicity were asymptomatic at the time of examination and total blood Pb levels in this group ranged from 50 to 75 μg%.

Erythrocytes were separated from plasma by centrifuging heparinized blood at 3000 X *g* for 15 min. The red blood cells were washed and then hemolyzed by freezing and thawing. A 1-ml portion of hemolysate and 2.0 ml Tris buffer (0.05 *M*, pH 7.4) were centrifuged at 20,000 X *g* for 20 min to remove the membrane fraction, and the supernatant was applied to a 90 X 1.5 cm Sephadex G-75 column (void volume $V_0 = 75$ ml) and eluted with Tris buffer at 12 ml/h. Four fractions were collected. The first ("high-molecular-weight") fraction extended from the void volume to the first appearance of hemoglobin ($V_E/V_0 = 1.0$–1.3). The second fraction consisted of hemoglobin, which was detected by appearance of red color and maximum absorbance at 545 nm ($V_E/V_0 = 1.3$–1.5). The third fraction contained the protein of molecular weight 10,000 in an elution volume encompassed by $V_E/V_0 = 1.8$–2.1. The fourth fraction ("free Pb") extended from $V_E/V_0 = 2.1$–2.5 and presumably contained both inorganic Pb and Pb bound to amino acids or small peptides. Succeeding fractions contained no detectable Pb. The Pb was measured in lyophilized fractions by use of an atomic absorption spectrophotometer (AAS) with a graphite furnace attachment and the Pb content of each fraction was expressed as micrograms per 100 ml original hemolysate.

As indicated above, the membrane fraction from erythrocytes was obtained by centrifugation at 20,000 X *g* for 15 min. Membranes were washed twice with isotonic saline and dissolved in 0.1% Triton X-100. The Pb content was measured by AAS and expressed as micrograms per 100 ml original hemolysate. Activity of Na^+,K^+-ATPase was measured in the membrane fraction by the method of Hasan et al. (1967b) and expressed as micromoles of inorganic phosphate (P_i) per milligram of protein per hour. A 1-ml portion of heparinized blood was mixed with 5 ml 0.15 *M* NaCl and the cells were centrifuged at 15,000 X *g* for 10 min. After removal of the plasma layer and buffy coat, the packed erythrocytes were washed twice in 0.15 *M* NaCl, then hemolyzed by rapidly adding 5 ml distilled water. The sediment from further centrifugation at 20,000 X *g* was suspended in 1 ml 0.1 *M* histidine-imidazole buffer, pH 7.1, and a 0.1-ml portion was taken for Na^+,K^+-ATPase assay. Activity of the enzyme was measured as the difference between P_i liberated in the presence and in the absence of 10^{-3} *M* ouabain, with appropriate controls. All samples were run in duplicate; results agreed to ±5%.

RESULTS

The Pb content of the red blood cells, hemoglobin fraction, molecular weight 10,000 fraction, high-molecular-weight fraction, and membrane fraction in the normal controls and three groups of Pb workers is shown in Table 1. Total recovery of Pb summated from the individual fractions averaged 107% of the original red blood cell hemolysate Pb value; this verifies the accuracy of the determinations as well as our prior prediction that the calculated residual Pb would be found in the high-molecular-weight and membrane fractions. In the normal control population, the Pb content of the latter fractions averaged 5 μg%, accounting for 12% of the total red blood cell hemolysate Pb. In Pb workers without toxicity, the Pb in the high-molecular-weight fraction averaged 10 μg% or 7% of the total red blood cell hemolysate Pb and the lead in the membrane fraction averaged 12 μg% or 8% of the total red blood cell hemolysate Pb. In Pb workers with toxicity at either high or low total blood levels, the high-molecular-weight fraction averaged 32 μg% or 22% of the total red blood cell. The lead in the membrane fraction averaged 21 μg% or 15% of the total red blood cell.

Membrane Na^+,K^+-ATPase activity averaged 0.34 μmol/mg·h in normal controls, 0.23 μmol/mg·h in Pb workers without toxicity, and 0.11 μmol/mg·h in Pb workers with toxicity (significantly different from both controls and Pb workers without toxicity at $p < 0.05$).

Figure 1 shows the relation between total red blood cell Pb and membrane Na^+,K^+-ATPase activity, and Fig. 2 shows the relation between membrane Pb and membrane Na^+,K^+-ATPase activity. The correlation between total red blood cell Pb and membrane Na^+,K^+-ATPase activity was not significant ($r = -0.039$), whereas that between membrane Pb and the enzyme activity was highly significant ($r = -0.887$, $p < 0.001$).

DISCUSSION AND CONCLUSIONS

Toxic effects of Pb on the hematopoietic system are exhibited by defects in heme and globin synthesis, with resultant retardation of erythrocyte maturation, and by hemolysis of circulating mature erythrocytes (Goldberg, 1968; White and Harvey, 1972; Beck et al., 1970; Hasan et al., 1967*a*). Hemolysis is probably related in part to inhibition of erythrocyte membrane Na^+,K^+-ATPase activity, resulting in premature lysis of affected cells on an osmotic basis (Bonting and Caravaggio, 1963). Premature hemolysis may also be related to inhibition by Pb of pyrimidine 5′-nucleotidase, an enzyme found in the cytosol of mature erythrocytes, as shown by Paglia et al. (1975). Treatment of Pb-intoxicated individuals with EDTA rapidly reverses the hemolytic component, but not the effect of Pb on heme biosynthesis (Beck et al., 1970). These observations suggest that there is a mobilizable fraction of Pb in one or more compartments of the mature erythrocyte that contributes to hemolysis by an effect on susceptible enzymes.

TABLE 1. Pb Content of Red Blood Cells and Red Blood Cell Pb-binding Fractions (μg%)

	Patient	Red blood cells	Hemoglobin	Molecular weight 10,000 fraction	High-molecular-weight fraction	Membrane fraction
			Normal Controls			
	1	60	34	2	6	5
	2	43	23	2	5	5
	3	52	26	3	5	5
	4	42	23	3	5	4
	5	46	22	2	6	5
	6	29	19	2	4	4
	7	32	19	2	4	4
	8	31	20	2	4	4
Mean ± SE		42 ± 4	23 ± 2	2 ± 0.2	5 ± 0.3	5 ± 0.2
Percent of red blood cell Pb		100	55	5	12	12
			Pb-exposed Workers without Toxicity			
	1	163	98	39	18	15
	2	141	83	37	14	10
	3	172	103	53	9	12
	4	157	92	36	14	17
	5	178	106	47	9	20
	6	128	81	30	6	11
	7	145	89	36	10	9
	8	145	98	21	14	13
	9	128	87	26	8	9
	10	102	65	28	6	7
	11	150	93	36	8	14
	12	107	68	26	6	10
	13	154	98	34	12	14
	14	124	79	33	8	9
Mean ± SE		142 ± 6	88 ± 3	34 ± 2	10 ± 1	12 ± 1
Percent of red blood cell Pb		100	62	24	7	8
			Pb-exposed Workers with Toxicity at High Blood Pb Levels			
	1	168	81	33	33	24
	2	208	102	42	38	26
	3	174	89	31	41	20
	4	192	96	38	44	28
	5	185	104	30	28	21
Mean ± SE		185 ± 7	94 ± 4	35 ± 2	37 ± 3	24 ± 2
Percent of red blood cell Pb		100	51	19	20	13
			Pb-exposed Workers with Toxicity at Low Blood Pb Levels			
	1	108	63	4	27	16
	2	120	72	5	29	18
	3	102	60	4	24	19
	4	110	58	4	24	20
	5	118	67	5	31	16
Mean ± SE		112 ± 3	64 ± 3	4 ± 0.2	27 ± 1	18 ± 1
Percent of red blood cell Pb		100	57	4	24	16

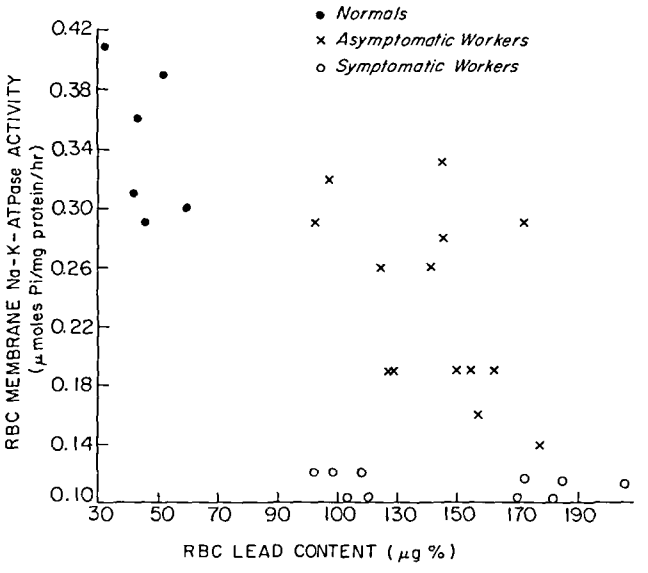

FIGURE 1. Relation between red blood cell membrane Na^+, K^+-ATPase activity and total red blood cell Pb content.

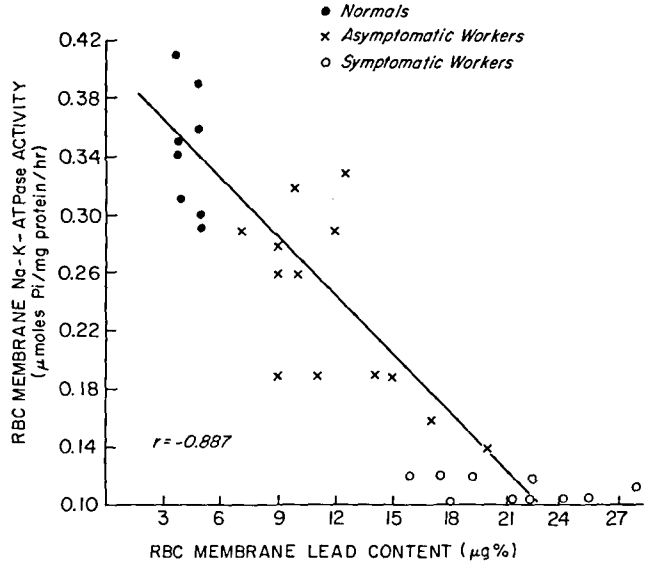

FIGURE 2. Relation between membrane Na^+, K^+-ATPase activity and red blood cell membrane Pb content.

In our initial study, we presented evidence for the presence of a Pb-binding protein of molecular weight 10,000 in erythrocytes from Pb workers. As this protein was not found in normal subjects, we speculated that its synthesis was induced in response to Pb exposure and that it might serve to segregate Pb in a nontoxic form. In a subsequent study, we defined a subgroup of industrial workers who were particularly susceptible to Pb intoxication because of their inability to bind Pb to this protein (workers with toxicity at low blood Pb levels). Reduced binding of Pb to this protein fraction resulted in accumulation of Pb in a residual fraction, which was defined as the difference between total red blood cell Pb and Pb in the hemoglobin fraction, the molecular weight 10,000 Pb-binding protein fraction, and a free Pb fraction. The present study extends these observations by demonstrating that the residual Pb in red blood cells is approximately evenly divided between Pb in a high-molecular-weight fraction, which elutes before hemoglobin in Sephadex G-75 gel chromatography, and membrane-bound Pb. Enzymes of molecular weight greater than 70,000, such as ALAD (250,000 daltons), should be present in the high-molecular-weight fraction and thus inhibited by the Pb segregated in this compartment. Likewise, Pb bound to the red blood cell membrane compartment would be available to inhibit the membrane transport enzyme Na^+,K^+-ATPase.

A decrease in red blood cell membrane Na^+,K^+-ATPase was reported in Pb workers with blood Pb ranging from 63 to 130 μg per 100 ml by Hasan et al. (1967b) and in a separate group of Pb workers with blood Pb ranging from 42 to 100 μg per 100 ml by Secchi et al. (1968). In both reports this decrease in Na^+,K^+-ATPase activity appeared to be a threshold effect, with no linear correlation between total blood Pb and enzyme activity. In the present study we confirmed the inhibition of membrane Na^+,K^+-ATPase in red blood cells of Pb-intoxicated workers and were able to demonstrate a highly significant inverse correlation between the enzyme activity and the fraction of Pb in the membrane compartment. These findings suggest that the primary determinant of Pb toxicity in a given cell is the distribution of Pb among nontoxic binding proteins and compartments that contain susceptible enzyme systems.

REFERENCES

Beck, P. D., Tschudy, D. P., Shipley, L. A., Waggoner, J. G., and Berlin, N. I. 1970. Hematologic and biochemical studies in a case of lead poisoning. *Am. J. Med.* 48:137–144.

Bonsignore, D., Calissano, P., and Cartasegna, C. 1965. Un semplice metodo per la determinazione della δ-amino-levulinico-deidratase nel sangue. *Med. Lav.* 56:199–205.

Bonting, S. L. and Caravaggio, L. L. 1963. Studies on sodium-potassium-activated ATPase. V. Correlation of enzyme activity with cation flux in six tissues. *Arch. Biochem. Biophys.* 101:37–43.

Goldberg, A. 1968. Lead poisoning and haem biosynthesis. *Semin. Hematol.* 5:424–433.

Haeger-Aronsen, B., Abdulla, M., and Fristedt, B. I. 1971. Effect of lead on δ-aminolevulinic acid dehydratase activity in red blood cells. *Arch. Environ. Health* 23:440–445.

Hasan, J., Hernberg, S., Metsala, P., and Vihko, V. 1967a. Enhanced potassium loss in blood cells from men exposed to lead. *Arch Environ. Health* 14:309–312.

Hasan, J., Vihko, V., and Hernberg, S. 1967b. Deficient red cell membrane Na-K-ATPase in lead poisoning. *Arch. Environ. Health* 14:313–318.

Hernberg, S. and Nikkanen, J. 1972. Effect of lead on δ-aminolevulinic acid dehydratase: A selective review. *Prac. Lek* 24:77–83.

Hernberg, S., Vihko, V., and Hasan, J. 1967. Red cell membrane ATPases in workers exposed to inorganic lead. *Arch. Environ. Health* 14:319–324.

Paglia, D. E., Valentine, W. N., and Dahlgren, J. G. 1975. Effects of low-level lead exposure on pyrimidine 5'-nucleotidase and other erythrocyte enzymes. Possible role of pyrimidine 5'-nucleotidase in the pathogenesis of lead induced anemia. *J. Clin. Invest.* 56:1164–1169.

Post, R. L., Merritt, C. R., Kinsolving, C. R., and Albright, C. D. 1960. Membrane ATPase as a participant in the active transport of sodium and potassium in the human erythrocyte. *J. Biol. Chem.* 235:1796–1802.

Raghavan, S. R. V. and Gonick, H. C. 1977. Isolation of low molecular weight lead-binding protein from human erythrocytes. *Proc. Soc. Exp. Biol. Med.* 155:164–167.

Raghavan, S. R. V., Culver, B. D., and Gonick, H. C. 1980. Erythrocyte lead-binding protein after occupational exposure. I. Relationship to lead toxicity. *Environ. Res.* 22:264–270.

Secchi, G. C., Rezzonico, A., and Alessio, L. 1968. Variazioni dell'activita' nelle diverse fasi dellintossicazione saturnina. *Lav. Med* 22:191–196.

White, J. M. and Harvey, D. R. 1972. Defective synthesis of α and β globin chains in lead poisoning. *Nature (Lond.)* 236:71–73.

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