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Isolation of Low-Molecular-Weight Lead-Binding Protein from Human Erythrocytes (39766)

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In blood, lead is mainly associated with erythrocytes and only a very small amount is found in plasma (1). Previously it was thought that the lead was bound to the erythrocyte cell membrane (2) but more recently it has been observed that lead is bound primarily to the cell contents, ostensibly hemoglobin (3). In examining the lead-binding properties of normal human erythrocytes and those of lead-exposed industrial workers, we have found that, whereas lead binds only to hemoglobin in normal erythrocytes, there is also appreciable binding of lead to a low-molecular weight-protein in erythrocytes from lead-exposed workers.

Materials and methods. Blood was collected in heparinized syringes from six men with symptomatic industrial lead intoxication and from three healthy males of comparable age who had no known exposure to lead. Erythrocytes (RBC) were separated from plasma by centrifuging the blood at 3000*g* for 15 min. The RBC were washed three times with normal saline and hemolyzed by freezing and thawing. To 1.0 ml of the hemolysate 2.0 ml of Tris buffer (0.05 *M*, pH 7.4) and 0.1 ml (containing 0.25 μCi) of ^{210}Pb nitrate (1 mCi/0.016 mg of Pb, Amersham/Searle) were added and incubated for 1 hr at room temperature. After incubation, the contents were centrifuged at 20,000*g* for 20 min. The membrane fraction was found to contain 16.5% of the total radioactivity; the remaining counts were recovered quantitatively from the supernatant (cytoplasm). An aliquot of the supernatant (0.2 ml) was taken out for lead determination and 1.0 ml from the remainder was counted and fractionated on a 90 × 1.5-cm Sephadex G-75 column (vol = 72 ml) at a rate of 12 ml/hr, using Tris buffer (0.05 *M*, pH 7.4) as eluate. The column was calibrated with ovalbumin[2] (mol wt 45,000) and ribonuclease[2] (mol wt 13,700). The locus of hemoglobin in the eluate was detected by appearances of red color and maximum absorbance at 545 nm. Four void volumes were collected and monitored for radioactivity. This was found to be concentrated in two fractions, corresponding to hemoglobin and a 10,000-mol-wt fraction (*vide infra*). Inorganic lead in whole RBC, the hemoglobin fraction and the 10,000-mol-wt fraction was determined by atomic absorption spectrophotometry with a graphite furnace. Radioactivity of ^{210}Pb was measured in a Packard γ-spectrometer. All samples were run in duplicate. Variability between duplicate samples was less than 3% for both inorganic lead and radioactivity. SDS-acrylamide gel electrophoresis was performed according to the method of Weber and Osborn (6) on aliquots of the hemolysates from normal and lead-exposed persons. In addition, gel electrophoresis was performed on the 10,000-mol wt protein fraction obtained through Sephadex gel filtration of hemolysates from the lead-exposed individuals.

Results. Following Sephadex gel filtration of hemolysates from the lead-exposed individuals and the normal controls the ^{210}Pb was found to be concentrated in two fractions corresponding to hemoglobin and a low-molecular-weight substance. Calibration of the column with ribonuclease indicated that the molecular weight of the unknown substances was approximately 10,000 (Fig. 1). In the lead-exposed individuals approximately 50% of the ^{210}Pb in the RBC cytoplasm could be recovered from the hemoglobin fraction and the

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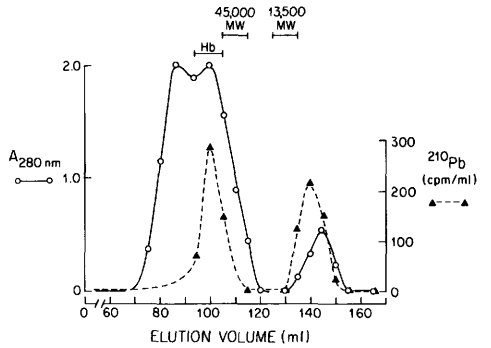

FIG. 1. Sephadex G-75 gel filtration of RBC hemolysate from lead-exposed individual. Ultraviolet absorption and radioactivity of ^{210}Pb are plotted against elution volume. The column was calibrated with ovalbumin (mol wt 45,000) and ribonuclease (mol wt 13,700). Also indicated is the locus of hemoglobin (Hb). Hemolysates from normal control individuals showed no uv absorption or radioactivity in the volume eluting between 130 and 155 ml.

10,000-mol-wt fraction, with two-thirds of the recovered isotope located in the hemoglobin (Table I). In the normal controls approximately 40% of the ^{210}Pb was recovered, all in the hemoglobin fraction. Measurements of lead concentration in RBC, hemoglobin fraction, and 10,000-mol-wt fraction are presented in Table II. The lead concentrations in RBC and the hemoglobin fraction are elevated approximately fivefold in lead-exposed patients as contrasted to the normal controls. In addition, as noted with ^{210}Pb, the total recovered lead in the cytoplasmic fraction of the RBC is distributed approximately two-thirds in the hemoglobin fraction and one-third in the 10,000-mol-wt fraction in the lead-exposed patients. In contrast, there is only an insignificant amount of lead found in the elution volume corresponding to the 10,000-mol-wt fraction in the normal controls. The acrylamide gel electrophoretic pattern of the hemolysates and the 10,000-mol-wt fraction is shown in Fig. 2. The 10,000-mol-wt fraction (tube C) produces two bands when stained with coomassie blue, but only the uppermost band contains lead. The same two bands are present in the hemolysate from the lead-exposed individuals (tube B) but not in the hemolysate from the normal controls (tube A). The 10,000-mol-wt band was identified as a protein by virtue of its ability to be stained by the coomassie blue and by its reaction with Biuret and Lowry reagents.

Discussion. In the RBC of normal control individuals approximately 60% of the lead present was found in the hemoglobin fraction. The remainder of the lead presumably was present as either membrane-bound lead, free lead, or bound to substances of molecular weight less than 1000. Hemolysates of RBC from lead-exposed individuals yielded a protein of approximately 10,000 mol wt, which was capable of binding lead to a significant degree. As this protein could not be detected in hemolysates of RBC from normal control individuals, it may be inferred that synthesis of this protein is induced by lead exposure. It has long been recognized that nuclear inclusion bodies are present in the kidney tubules from lead-exposed humans and experimental animals (7, 8). Goyer and his colleagues have dem-

TABLE I. PERCENTAGE OF ^{210}Pb FROM RBC CYTOPLASM IN HEMOGLOBIN AND 10,000-MOL-WT FRACTIONS.

Group of patients	Hemoglobin fraction	10,000-mol-wt fraction
Lead-exposed		
(1)	24	24
(2)	30	10
(3)	32	19
(4)	29	16
(5)	30	20
(6)	33	19
Normal controls		
(1)	38	0
(2)	39	0
(3)	40	0

TABLE II. LEAD CONCENTRATION IN RBC, HEMOGLOBIN AND 10,000-MOL-WT FRACTIONS.

Group of patients	RBC (μg%)	Hemoglobin fraction (μg%)	10,000-mol-wt fraction (μg%)
Lead-exposed			
(1)	209	98	53
(2)	185	109	28
(3)	162	74	58
(4)	205	106	31
(5)	164	97	45
(6)	166	126	21
Normal controls			
(1)	33	20	3
(2)	44	30	3
(3)	49	28	4

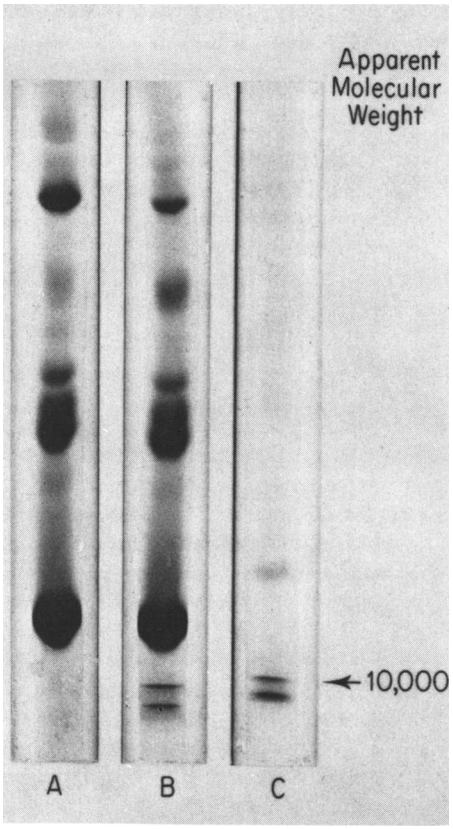

FIG. 2. SDS-polyacrylamide gel electrophoresis of RBC hemolysates from normal control (A) and lead-exposed individual (B), and of low-mol-wt. lead-binding protein (C). Stained with coomassie blue.

onstrated that the nuclear inclusion body is composed of lead in association with a 30,000-mol-wt protein, and they have suggested that this lead-protein complex may function as a store or depot for intracellular lead (9). We would propose that the 10,000-mol-wt. protein found in the RBC of lead-exposed patients also serves as an intracellular store and further may act as a mechanism for segregating lead in a nontoxic form.

In an analogous situation, cadmium exposure is known to induce the synthesis of a protein of approximately 10,000 mol wt named metallothionein because of its high content of sulfhydryl groups and metals, principally cadmium (10). This protein has been found in several organs of the cadmium-exposed animal, including liver, testis, pancreas, spleen, brain, kidney, and RBC, and is thought to play a protective role against the toxic effects of cadmium (10, 11). A 30,000-mol-wt protein with a higher cadmium-binding affinity than metallothionein has also been found in the testis of the rat and has been proposed as the molecular basis for the peculiar sensitivity of the rat testis to cadmium (11). Although there are insufficient data to state whether metallothionein and the 10,000-mol-wt lead-binding protein are the same, certain observations suggest that this is probably not the case. Analyses of the heavy metal content of metallothionein purified from rat liver have not revealed lead, although trace quantities of zinc, mercury, copper, silver, and tin have been found in addition to cadmium (10, 12). These observations have been confirmed by *in vivo* radiotracer experiments in which isotopes of the above metals, but not lead, were found to be incorporated into metallothionein (12).

Further comparisons between metallothionein and the 10,000-mol-wt lead-binding protein will only be possible when the latter protein has been purified and its amino acid and metal composition have been determined. Such studies are now in progress.

Summary. A low-molecular-weight lead-binding protein has been identified in erythrocytes from individuals with industrial exposure to lead. This protein is not present in erythrocytes from normal individuals.

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