

## Lead poisoning. Further observations on erythrocyte pyrimidine-nucleotidase deficiency and intracellular accumulation of pyrimidine nucleotides.

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### Research Article

Pyrimidine nucleotides, detectable in normal erythrocytes only in trace quantities if at all, were found to comprise 7-80% of the intracellular nucleotide pools in nine subjects with severe lead over-burden. Blood lead concentrations ranged from approximately equal to 200- to 400-microgram/dl packed cells, and the greatest accumulations of pyrimidine-containing nucleotides occurred in the two subjects with the highest blood lead levels. Most of the patients had mild or moderate anemia and moderate basophilic stippling evident in Wright's-stained peripheral smears. Pyrimidine nucleotidase activities were inhibited to 13-28% of the mean activity in normal control erythrocytes and even more so (5-15%) when compared to specimens with increased reticulocytes and young cells. Reticulocytosis was absent in two subjects and modest to moderate in the remainder, but erythrocyte assays revealed the substantial elevations in populations of young mean cell age. Inappropriately low reticulocytial elevations in glucose-6-phosphate dehydrogenase expected in populations of young mean cell age. Inappropriately low reticulocyte responses may reflect hematopoietic suppressive effects of lead at a variety of metabolic loci.

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# Lead Poisoning

## FURTHER OBSERVATIONS ON ERYTHROCYTE PYRIMIDINE-NUCLEOTIDASE DEFICIENCY AND INTRACELLULAR ACCUMULATION OF PYRIMIDINE NUCLEOTIDES

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**ABSTRACT** Pyrimidine nucleotides, detectable in normal erythrocytes only in trace quantities if at all, were found to comprise 7–80% of the intracellular nucleotide pools in nine subjects with severe lead overburden. Blood lead concentrations ranged from  $\approx 200$ - to  $400\text{-}\mu\text{g/dl}$  packed cells, and the greatest accumulations of pyrimidine-containing nucleotides occurred in the two subjects with the highest blood lead levels. Most of the patients had mild or moderate anemia and moderate basophilic stippling evident in Wright's-stained peripheral smears. Pyrimidine nucleotidase activities were inhibited to 13–28% of the mean activity in normal control erythrocytes and even more so (5–15%) when compared to specimens with increased reticulocytes and young cells. Reticulocytosis was absent in two subjects and modest to moderate in the remainder, but erythrocyte assays revealed the substantial elevations in glucose-6-phosphate dehydrogenase expected in populations of young mean cell age. Inappropriately low reticulocyte responses may reflect hematopoietic suppressive effects of lead at a variety of metabolic loci.

### INTRODUCTION

The nucleotidase (5'-ribonucleotide phosphohydrolyase, EC 3.1.3.5) present in normal human erythrocytes is highly specific in that it functions only with pyrimidine and not purine nucleotide substrates. Severe

hereditary deficiency of this enzyme is associated with a syndrome consisting of hemolytic anemia, markedly increased basophilic stippling of erythrocytes on the stained blood film, elevated erythrocyte reduced glutathione (GSH),<sup>1</sup> and uniquely, large accumulations of intraerythrocytic cytidine- and uridine-containing nucleotides (1, 2). Pyrimidine nucleotides resulting from RNA degradation in the maturing reticulocyte are normally rendered diffusible by nucleotidase-mediated dephosphorylation, but accumulate in cells with insufficient nucleotidase activity (1). The prominent basophilic stippling is thought to reflect impaired ribosomal degradation resulting from feedback inhibition by pyrimidine-containing end products. The syndrome has been documented by investigators in France (3), Israel (4), Spain (5), and Japan<sup>2</sup> as well as in other laboratories in the United States (6, 7).

The similarity of the hereditary syndrome to the anemia and prominent basophilic stippling characteristic of certain cases of severe lead intoxication, and the *in vitro* sensitivity of erythrocyte pyrimidine nucleotidase to low concentrations of lead, prompted our initial investigation of nucleotidase activities in 15 individuals with mild-to-moderate lead overburden (blood lead =  $45\text{--}110\ \mu\text{g/dl}$  whole blood) (8). Only one of these subjects was anemic and none exhibited basophilic stippling. Significantly diminished nucleotidase

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<sup>1</sup>Abbreviation used in this paper: GSH, reduced glutathione.

<sup>2</sup>Miwa, S. Personal communication.

activity was uniformly observed, but intracellular accumulations of pyrimidine nucleotides could not be demonstrated. It was postulated, however, that lead-induced nucleotidase deficiency, if sufficiently severe, would mimic the genetically determined disorder, including the intraerythrocytic accumulation of pyrimidine nucleotides. We have reported a single case of severe lead intoxication in which the findings verified the postulate (9). This subject had hemolytic anemia, reticulocytosis, marked basophilic stippling, and  $\approx 15\%$  of normal pyrimidine nucleotidase activity. 12% of the erythrocyte nucleotides was cytidine phosphates, however, all disappeared after occupational exposure to toxic concentrations of lead was discontinued. The present report documents nine additional cases of severe lead intoxication with greatly diminished erythrocyte nucleotidase activity and intracellular accumulations of pyrimidine nucleotides.

## METHODS

The methods employed in these studies were identical to those presented in detail in our earlier report (9). Because blood lead concentrations essentially reflect those of the erythrocyte and very little lead is found in plasma (10), the values recorded in Table I are expressed in micrograms per deciliter of packed erythrocytes. In contrast, most commercial laboratories report blood lead concentrations in micrograms per deciliter of whole blood, a misleading representation of the actual erythrocyte burden when anemic and nonanemic subjects are compared. The values recorded here have been computed from whole blood lead concentrations or from

TABLE I  
*Hematologic and Lead-Assay Values in Nine Subjects with Lead Toxicity*

| Patient          | (Sex) | Packed cell volume | Hemoglobin  | Reticulocytes | Blood lead*               | Urine lead     |
|------------------|-------|--------------------|-------------|---------------|---------------------------|----------------|
|                  |       | <i>ml/dl</i>       | <i>g/dl</i> | <i>%</i>      | <i>μg/dl packed cells</i> | <i>μg/24 h</i> |
| Kos              | (M)   | 38.0               | 12.9        | 4.9           | 253                       | 659            |
| Re               | (M)   | 34.0               | 9.3         | 1.0           | 238                       | 230            |
| D-Sa             | (F)   | 26.6               | —           | 7.8           | 338                       | 281            |
| Sl               | (F)   | 24.0               | 7.9         | 3.0           | 236                       | 1,085          |
| Koc              | (M)   | 48.5               | 15.5        | 1.1           | 198                       | 100            |
| Pi               | (M)   | 44.0               | 13.4        | 2.7           | 200                       | 219            |
| Pr               | (M)   | 42.0               | 13.3        | 2.8           | 276                       | 412            |
| Ra               | (M)   | 40.5               | 13.0        | 3.7           | 416                       | 192            |
| Si               | (M)   | 44.0               | 15.0        | 2.9           | 208                       | 302            |
| Normal controls: |       |                    |             | <1.0          | <60                       | <100           |

\* In the first four cases, whole-blood lead concentrations were converted to the recorded values on the basis of the packed cell volume of each specimen. In the last five cases, erythrocytes were washed with saline and lead concentrations expressed per 100 ml of packed erythrocytes.

saline-washed erythrocyte suspensions, and converted to packed cells on the basis of the packed erythrocyte volume of each specimen. Whereas "normal" erythrocyte lead values are controversial and obviously vary with environmental exposure to lead in any given subject, we have arbitrarily accepted 60- $\mu\text{g}/\text{dl}$  packed cells as an upper limit. This is in accord with the criteria of Dr. John Hines<sup>3</sup>, Western Reserve School of Medicine, Cleveland, Ohio, who kindly provided specimens of blood from five of the nine subjects with lead overburden reported here.

## Case material

Seven males had heavy occupational exposures to fumes and dust in industrial operations involving lead. The first exposure of one female subject (Sl) was related to drinking acidic beverages from pottery coated with lead-containing glaze. The source of a second exposure in this subject has not yet been determined. In the other female subject (D-Sa), intoxication was secondary to chronic oral ingestion of large amounts of lead in exotic herbal medications, the details of which have been reported elsewhere (11). Symptoms and clinical findings varied, but common complaints were fatigue, arthralgia, myalgia, irritability, and abdominal pain. Other symptoms included impotence, dizziness, nausea, anorexia, insomnia, decreased powers of concentration, paranoid mentation, and manifestations referable to peripheral neuropathy. The laboratory documentation of lead overburden and hematologic findings are recorded in Table I.

## RESULTS

*Hematologic values.* Hemoglobin values were normal in two of the nine subjects. The other seven, however, were anemic with hemoglobin concentrations of <13.5 g/dl (Table I). In two of these, anemia was severe with 24- and 27-ml/dl packed cell volumes. Reticulocytes were not significantly increased in two instances and ranged from  $\approx 3$  to 5% in the others, except for one which was almost 8%. In all subjects, the Wright's-stained peripheral blood smear revealed increased basophilic stippling of erythrocytes, moderate increases being the rule but with very marked stippling in the cells of subject D-Sa and mild stippling in subject Re.

*Lead toxicity studies.* The marked elevations in erythrocyte lead present in all subjects is also recorded in Table I. Urinary lead concentrations were also substantially increased, varying from upper limits of normal to more than 10-fold normal. Other indicators of lead toxicity (not tabulated) were obtained in most instances and confirmed the presence of large tissue burdens. Blood specimens obtained by Dr. John Hines, those of the last five subjects of Table I, exhibited free erythrocyte protoporphyrin concentrations  $\approx 15$ –20 times normal, whereas ratios of deactivated to activated  $\delta$ -aminolevulinic acid dehydratase were increased  $\approx 5$ –8 times.<sup>3</sup> These findings are strongly indicative

<sup>3</sup> Hines, J. Personal communication.

of significant lead overburdens of relatively long-standing. In other subjects, urinary  $\delta$ -aminolevulinic acid and coproporphyrin were substantially increased, and in the first four patients recorded in Table I, treatment with calcium EDTA was followed by severalfold increases in daily urinary lead excretion.

**Erythrocyte nucleotides and nucleotidase activities.** Table II records pyrimidine nucleotidase activity in enzyme units and the percentage of total erythrocyte nucleotides containing adenosine, cytidine, and uridine, respectively. In all subjects nucleotidase activity was greatly depressed, ranging from 13 to 28% of the normal mean with UMP and from 10 to 27% with CMP as substrate. If comparison is made to reticulocyte-rich blood, in which nucleotidase activity is characteristically increased (1, 2), the enzymatic defect is even more profound. Although reticulocyte counts were modestly to moderately elevated, even normal in two instances, there was ancillary evidence that the blood samples were composed of younger than normal cells. In seven of eight subjects where assay of erythrocyte glucose-6-phosphate dehydrogenase activity was performed, the values were  $<3$  SD above the normal mean. Increased glucose-6-phosphate dehydrogenase activity is regularly observed in erythrocyte populations of young mean cell age and diminishes in activity as the erythrocytes age.

In eight subjects, cytidine and uridine nucleotides

comprised 7–27% of total cell nucleotides. Normally, pyrimidine-containing nucleotides are present in vanishingly small amounts in either normal or reticulocyte-rich erythrocyte populations (12–20) and are undetectable by the methods (21, 22) employed in our laboratory. In one patient (D-Sa), pyrimidine nucleotides constituted 80% of the total nucleotides present, a percentage comparable to that observed in homozygotes with genetically induced nucleotidase deficiency. This subject had the second highest blood lead concentration in this series and also had the most severe clinical manifestations of lead toxicity observed in any of the patients studied.

**Erythrocyte GSH.** For reasons not yet apparent, elevated erythrocyte GSH is characteristic of severe, genetically determined pyrimidine nucleotidase deficiency, presumably as an epiphenomenon. This has been observed in only a single subject with lead-induced deficiency (patient Pi, erythrocyte GSH =  $1,292 \mu\text{g}/10^{10}$  cells, normal =  $740 \pm 110$ ).

**Ribosephosphate pyrophosphokinase.** The hereditary nucleotidase defect is also associated with a significant but partial deficiency ( $\approx 30\%$ ) of erythrocyte ribosephosphate pyrophosphokinase (5-phosphoribosyl-1-pyrophosphate synthetase, EC 2.7.6.1) (1). None of the subjects reported herein, nor any of the lead-toxic subjects reported previously (8, 9) has exhibited any abnormality in this erythrocyte enzyme.

TABLE II  
*Erythrocyte Nucleotides and Pyrimidine Nucleotidase Activities in Nine Subjects with Lead Toxicity*

| Patient  | Adenosine* | Cytidine | Uridine | Cytidine + uridine | Pyrimidine nucleotidase† |                   |
|--|------------|----------|---------|--------------------|--------------------------|-------------------|
|  |            |          |         |                    | UMP                      | CMP               |
| % of total intracellular nucleotides             |            |          |         |                    | EU                       | EU                |
| Kos  | 93.0       | 7.0      | 0.0     | 7.0                | 2.4                      | 1.5               |
| Re   | 88.0       | 12.0     | 0.0     | 12.0               | 1.4                      | 0.7               |
| D-Sa   | 19.4       | 50.4     | 30.2    | 80.6               | 2.7                      | 1.5               |
| Sl   | 88.6       | 4.8      | 6.6     | 11.4               | 1.5                      | 1.1               |
| Koc  | 80.9       | 2.2      | 16.9    | 19.1               | 2.8                      | 1.9               |
| Pi   | 89.4       | 1.4      | 10.2    | 11.7               | 2.8                      | 2.0               |
| Pr   | 87.0       | 7.6      | 5.4     | 13.0               | 1.3                      | 0.8               |
| Ra   | 75.0       | 10.8     | 14.2    | 25.0               | 2.0                      | 1.5               |
| Si   | 84.8       | 5.1      | 10.1    | 15.2               | 1.6                      | 0.7               |
| Normal ( $n = 27$ )                              | 100.0      | 0        | 0       | 0                  | 9.9<br>(SD = 2.5)        | 7.4<br>(SD = 1.8) |
| High reticulocyte controls<br>(4–41%, $n = 13$ ) | 100.0      | 0        | 0       | 0                  | 18.9                     | 13.5              |

The methods of blood sample collection used by various contributors precluded accurate quantitative measurements of total nucleotide concentrations. Values expressed as percentages were derived from chromatographic partitioning.

\* Values for adenosine include its deaminated derivative, inosine.

† Nucleotidase activities are expressed in enzyme units (EU) equivalent to micromoles of inorganic phosphorus liberated per hour per gram of hemoglobin at 37°C using UMP or CMP as substrates (2).

## DISCUSSION

These studies provide strong confirmatory evidence that lead-induced deficiency of erythrocyte nucleotidase closely resembles the syndrome associated with the hereditary deficiency state including its unique feature, accumulation of pyrimidine nucleotides within affected erythrocytes. The latter phenomenon has been reported only in homozygously inherited nucleotidase deficiency (1, 3-7) and in one case of severe lead toxicity (9). Individuals exposed to lower levels of lead and those who are heterozygous for the hereditary deficiency both may have significantly reduced nucleotidase activities but do not have demonstrable intracellular pyrimidines (1, 8). Whereas variably diminished erythrocyte nucleotidase activity is characteristic of lead intoxication in general, accumulation of pyrimidines apparently occurs only when lead overburden and the resultant enzyme deficiency are severe. From the data presented here and in our previous reports (8, 9) it would appear that lead concentrations in the region of 200- $\mu\text{g}/\text{dl}$  packed erythrocytes are required before intracellular pyrimidine nucleotides are readily detectable.

It should be emphasized that assays of erythrocyte lead content and of nucleotidase activities both represent mean values for a large population of cells of varying ages. It is entirely possible that both measurements may differ significantly among various cohorts of cells making up the total population. In addition, accumulation of pyrimidine nucleotides is possible only during a very brief period in the life of the non-nucleated erythrocyte, namely, that period during which RNA degradation is occurring as the reticulocyte matures. After these degradative products have been cleared from the cell, the enzyme no longer has a function. Therefore, nucleotidase inhibition in a sufficiently matured erythrocyte would not result in pyrimidine nucleotide build-up and basophilic stippling. For these reasons, one would not expect rigid correlations among blood lead concentrations, nucleotidase activities, and concentrations of pyrimidine nucleotides, especially in severe intoxications such as these in which the nucleotidase is probably maximally depressed. A general correlation is evident, however, in that pyrimidine nucleotides accumulate only when blood lead concentrations are high and when nucleotidase activities are very low. The two subjects with the highest blood lead concentrations showed the most significant ratios of pyrimidine to adenine nucleotides. 80% of the erythrocyte nucleotide pool in one of these cases (D-Sa) consisted of pyrimidine nucleotides, about the same percentage observed in cells of homozygotes with genetically determined nucleotidase deficiency.

These general correlations are evident in Fig. 1 which shows nucleotidase activity as a function of

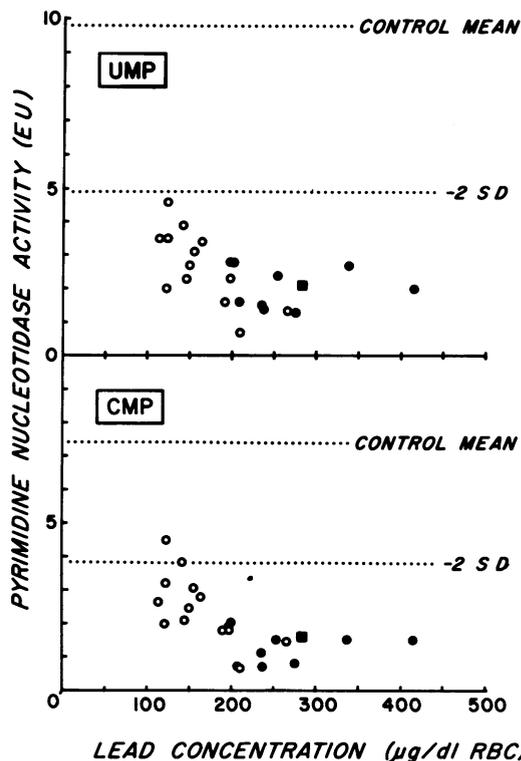


FIGURE 1 Pyrimidine nucleotidase activities with uridine and cytidine 5'-monophosphate substrates (UMP and CMP) as a function of lead concentration in erythrocytes (RBC) from subjects with varying degrees of lead overburden. Open symbols are from individuals with chronic low-level lead exposures and no evidence of intracellular pyrimidines (8). Solid symbols represent cases of acute lead toxicity including one previously reported (9), indicated by  $\blacksquare$ , all of whom had demonstrable intraerythrocyte pyrimidine nucleotides. Means and standard deviations depicted are based on current laboratory values, and each point is the mean of at least two determinations. Correlation coefficients using UMP and CMP as substrates were  $-0.5$  and  $-0.7$ , respectively.

erythrocyte lead content in 23 of the 25 subjects presented in this and in our previous studies (8, 9). (There were insufficient data to convert lead levels to packed cell concentrations in two of the chronically exposed individuals [8].) From these data, it is apparent that nucleotidase activity is progressively inhibited as erythrocyte lead concentrations increase until the latter reaches  $\approx 200\text{-}\mu\text{g}/\text{dl}$  packed cells, at which point the enzyme activity appears to be maximally depressed. Although this is far from a precise threshold, it is about the same point that intracellular pyrimidines become readily detectable.

Unlike hereditary nucleotidase deficiency where a single molecular lesion is implicated, lead affects mature erythrocytes and their precursors at multiple metabolic loci. Lead-induced nucleotidase deficiency is thus one of several pathogenetic mechanisms contributing to biochemical aberrations in the cell and

consequent hematologic abnormalities. The anemia of lead overburden may also be associated with ineffective erythropoiesis and impaired, inappropriately low, reticulocyte responses. With the exception of patient D-Sa reported here and in our earlier reported case (9), marked reticulocytosis has not been a consistent feature of the lead-induced deficiency. Nonetheless, the markedly increased glucose-6-phosphate dehydrogenase activities indicated that peripheral erythrocytes in these subjects had a considerably younger mean cell age than normal populations.

The percentage of erythrocytic pyrimidine-containing nucleotides in cases of lead toxicity, with one exception (D-Sa), has been much lower than in the genetically determined syndrome. In the latter, partial deficiency of the activity of ribosephosphate pyrophosphokinase (PRPP synthetase) and increased erythrocyte GSH concentrations have consistently been observed as unexplained epiphenomenon. At present, neither has been observed in the cells of subjects with lead overburden, with the single exception of an increased concentration of GSH in patient Pi.

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