Screening for Lead Poisoning with the Urinary ALA Test

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THE observation that elevated urinary concentrations of delta-aminolevulinic acid (ALA) are directly correlated with an excess body burden of lead in laboratory rabbits, industrial workers exposed to lead fumes (1), and children hospitalized with lead intoxication (2) suggested a simple, rapid, quantitative test for lead poisoning. The double ion-exchange column technique perfected by Davis and Andelman (3) made rapid determination of urinary ALA practicable.

To assess the value of this technique as a screening method, demonstrate the feasibility of employing local teenagers in such a ghetto health project, and determine the prevalence of abnormal ingestion of lead in high-risk slum children, we organized a door-to-door canvass in the Bronx, N.Y., in July and August 1968. Although both the collection and the assay methods proved workable, the reliability of the ALA test was reduced by the variable dilution of the urine specimens.

Method

Canvassers. Twelve local high school students, hired through community agencies, served as screening technicians. During a brief orientation they learned the causes of pediatric lead poisoning and the details of our approach to the problem. Since we believed local residents would be most likely to open their doors to girls but that unaccompanied girls might be insecure entering strange buildings, we paired boys and girls, with each couple including at least one Spanish-speaking partner. Each couple took responsibility for one block at a time and conducted a door-to-door canvass, making as many return visits as necessary. One of us (the three authors) remained at a prearranged spot in the area to supervise and coordinate the work.

Participation. The canvassers asked all parents with children 6 years of age or younger to cooperate. They briefly explained that many children sometimes chew on paint chips containing

lead and that once a child has become obviously sick he might be permanently disabled. They said that a specimen of each child's urine would be analyzed free at the medical school by a new technique. If the results suggested lead ingestion, the child would be referred to a local hospital for further testing. Nearly all the parents who listened to this explanation agreed to cooperate.

A few older children, siblings of those in the target group, asked or were told by their parents to give specimens, and data on them were included. Very young children (under 2 years old) from whom sample collection was difficult, are somewhat under-represented. Representation of children 2 through 6 years old, the group at highest risk, is fairly even (table 1), with roughly equal representation of both sexes.

Participation was obviously incomplete, but bias introduced by the sampling methods was difficult to assess. On the one hand, the sample may have included a relatively large number of conscientious, health-conscious families while excluding children left alone at home and those whose parents were too indifferent or suspicious to avail themselves of community services. On the other hand, the sample also excluded those families with children attending day-care facilities while the parents worked. Whether the net effect was a distortion toward higher or lower incidence of lead ingestion, we cannot estimate.

Study areas. Three neighborhood areas were selected for study.

1. Bronx census tract 77, bounded by Westchester and Prospect Avenues on the east and Jackson Avenue on the west, between 161st and 156th streets. This area consists of 12 blocks, several of which in 1968 were scheduled for demolition and were partly deserted. According to the 1960 census (*4*), the area had a population of 6,079 including 670 children under 5 years of age. The residents were predominantly Puerto Rican, with some Negro families. In 1960, the census classified 1,150 of the 1,650 housing units as deteriorating or dilapidated (*5*). Urine specimens from 138 children in this area were collected and analyzed.

2. Bronx census tracts 139, 141, and 143, bounded by Third Avenue on the east and Park and Clay Avenues on the west, between 167th and 161st streets. This area comprises 38 blocks of mixed manufacturing and residential buildings. The population in 1960 was 13,105 including 1,621 children under 5 years old (*4*). There were

4,320 housing units, 960 of which were classified as substandard (*5*). The population in 1968 was partly Puerto Rican and partly Negro. Specimens from 193 children were collected and analyzed.

Areas 1 and 2 were selected for study because readily available demographic data indicated a high proportion of members of minority groups with low incomes in the populations, unusually low average rents, overcrowding (a large number of persons per room), and, most significant, an extremely high proportion of deteriorating or dilapidated housing units. Records of the New York City Department of Health showed that these areas, although within the "lead belt" in which most reported cases of pediatric lead intoxication occurred, were not centers of peak incidence.

3. The third area, part of Bronx census tract 147, was used as a control. It is a few blocks north of areas 1 and 2. It consisted of part of the Daniel Webster Houses, a public housing project between Park and Webster Avenues and 169th and 170th Streets, which opened in 1965. The population was predominantly Puerto Rican and Negro, with a few white families. This area was selected as a control because it was comparable in terms of demographic and environmental variables to areas 1 and 2 but contained housing entirely free of lead-based interior paint. Samples from 59 children were collected and analyzed.

Sample collection and analysis. In all areas, the samples were collected between 10 a.m. and 3:30 p.m. Outside temperatures were in the 80° to 90° range. No attempt was made to record a child's recent intake of fluid. The canvassers were asked to complete a questionnaire on the general condition of each apartment and on the general health of each child, but these forms were often neglected.

Urine samples from older children were collected in paper cups or standard containers for specimens, and approximately 4 ml. aliquots were immediately transferred to labeled polyethylene tubes, which were sealed and returned to the laboratory. Pediatric bags were used for collecting specimens from younger children, and the samples were transferred to standard tubes in the laboratory. All samples were carried in the dark and were returned to the laboratory during the afternoon of collection and refrigerated at 4° C. in the dark. If the collecting devices were left at the homes, the parents were requested to refrigerate the samples until the team returned to pick them up the next day. We were unable to find any

evidence in the analyses that the specimens were contaminated by the collecting equipment.

When the samples were returned to the laboratory, they were tested for pH; any that were basic were acidified with acetic acid. Analysis for ALA content was performed by the method of Davis and Andelman (3). Disposable ion-exchange columns were obtained through the courtesy of the Bio-Rad Corporation of Richmond, Calif. The samples were analyzed 1 to 25 days after collection. ALA is stable for at least 15 days at pH of 4 to 7 and temperature below 4° C. (1). There was no trend and no significant deviation in the results of analyses grouped by the days elapsed between collection and analysis.

Creatinine (CR) content of the samples was determined by using the autolyzer of the surgical unit of the Montefiore Hospital and Medical Center. The reported values have an error of less than 5 percent.

Blood lead. Children with high urinary ALA levels and their siblings were referred for further examination to the Comprehensive Child Care Project of New York or to the pediatric services of Lincoln or Morrisania hospitals. None of these children showed clinical evidence of lead poisoning. We were able to obtain the results of blood lead determinations for 30 children whose ALA levels covered virtually the full range of all the children surveyed. Blood specimens were obtained 1 to 6 weeks after collection of the urine specimens.

Blood lead content was determined in the laboratory of the New York City Department of Health. The department uses the Dithizone extraction method and reports results in milligrams of lead per 100 milliliters to the second decimal place. The estimated error is less than 0.01 mg. per 100 ml.

Results

Ninety-five samples were omitted from our tabulations because of inadequate identifying information, insufficient quantity, or inadvertent storage unsealed or at pH greater than 7. The results reflect analyses of 390 samples unless otherwise noted. Because there was no evidence of a relationship between urinary ALA concentration and sex of the subject, analysis of the data by sex was not reported.

The mean urinary ALA concentration was 0.35 mg. per 100 ml. with a standard deviation of 0.24 mg. per 100 ml. Davis and co-workers (6) have

suggested that a urinary ALA concentration of more than 0.55 mg. per 100 ml. should be classified as a trace level. Haeger-Aronsen (1) reported that this level is roughly two standard deviations higher than the mean value in normal adults. Distribution of urinary ALA concentration by age in each of the three geographic areas, excluding 22 children for whom age was not recorded, is shown in table 1.

The mean concentration in area 1 was 0.41 mg. per 100 ml., in area 2, 0.34 mg. per 100 ml., and in the control area, 0.23 mg. per 100 ml. It is striking that not only did the means of areas 1 and 2 differ significantly from the mean of the control area (area 1, $P<0.00001$, and area 2, $P<0.002$), but that they also significantly differed from each other ($P<0.01$).

The prevalence of trace ALA concentrations varied significantly not only from area to area but also from building to building. In half of the 24 buildings in which five or more children were studied, there were no children with trace ALA levels; in seven, one or two youngsters showed trace levels, while in two buildings a majority of the children tested (five of eight in both buildings) showed urinary ALA in the trace range. The association with frank lead poisoning suggested by these data is supported by the reports of several parents in these two buildings that one or more of their children had been hospitalized for treatment of plumbism.

The prevalence of trace levels in areas 1 and 2

Table 1. Urinary delta-aminolevulinic acid (ALA) concentration in 368 children, by area of residence and age group

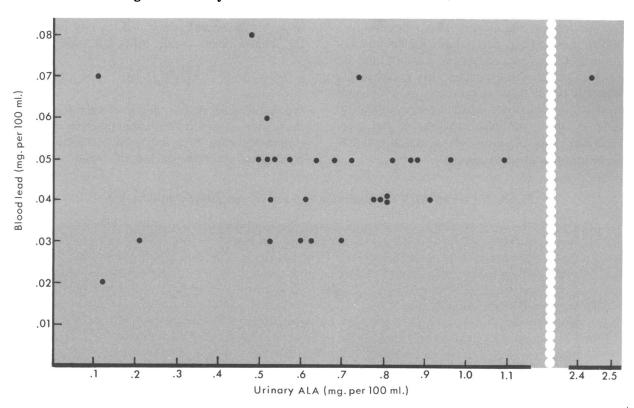| Area and age group (years)	Number of children	Urinary concentration, mg. per 100 ml.		
		ALA <0.55	0.55 ≤ ALA <0.95	0.95 ≤ ALA
Area 1:				
Over 6............	10	8	2	....
5–6..............	44	29	15	....
3–4..............	38	28	7	3
2 or under........	30	25	11	....
Area 2:				
Over 6............	12	10	1	1
5–6..............	54	49	6	....
3–4..............	75	65	9	1
2 or under........	47	45	2	....
Control area:				
Over 6............	5	5	......	....
5–6..............	20	20	......	....
3–4..............	23	23	......	....
2 or under........	10	10	......	....

Figure 1. Urinary ALA concentration and blood lead concentration

generally increased with age up to 5 years; the highest levels were seen in 3- and 4-year-olds, with one exception. Because lead poisoning is associated with older deteriorating housing, because ALA excretion remains elevated for at least 1 year after exposure to lead (2), and because the incidence of lead poisoning peaks at about age 3, all these results revealed a pattern consistent with what would be expected if an association existed between elevated urinary excretion of ALA and lead poisoning.

We could not demonstrate, however, that a significant relationship existed between urinary ALA concentration and blood lead concentration in the 30 children for whom data on blood lead content were available. Figure 1 shows the relationship between these parameters. The reported blood lead values ranged from 0.02 to 0.08 mg. per 100 ml., with a mean of 0.047 mg. per 100 ml. This relatively high mean is consistent with the selection for followup of youngsters drawn primarily from the group with high ALA levels. Mean urinary ALA for the group was 0.70 mg. per 100 ml., with standard deviation of 0.40 mg. per 100 ml. The coefficient of correlation between urinary ALA and blood lead was 0.3, which is not statistically significant with this size group ($P>0.10$).
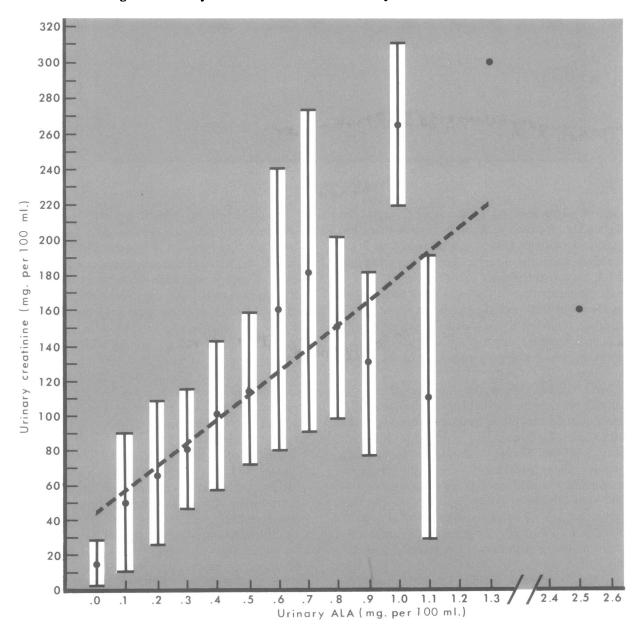
The child with the highest reported blood lead concentration (0.08 mg. per 100 ml.) had a urinary ALA concentration of less than 0.55 mg. per 100 ml. and was referred only because he was the sibling of a child with high ALA, while one child with blood lead of 0.07 mg. per 100 ml. had a urinary ALA value of less than 0.15 mg. per 100 ml. The rate of false negatives (that is, ALA less than 0.55 mg. per 100 ml. and blood lead greater than or equal to 0.06 mg. per 100 ml.) was 10 percent. The rate of false positives, even excluding borderline blood lead values (that is, ALA equal to or greater than 0.55 mg. per 100 ml. and blood lead equal to or less than 0.04 mg. per 100 ml.) was also 10 percent.

Despite the objections raised by Cramer and Selander (7), the urinary CR concentration is widely accepted as a reasonable index of urine concentration. The mean CR content of the 390 urine specimens was 92 mg. per 100 ml. The standard deviation was 60 mg. per 100 ml. The relationship between urinary ALA and CR concentrations in the 390 samples is shown in figure 2. The coefficient of correlation is 0.55, which, being statistically significant ($P<0.01$), suggests that variable dilution of random urine samples markedly influences ALA concentration. In the

two outstanding false negatives noted in figure 1, with blood lead concentrations of 0.08 and 0.07 mg. per 100 ml. and urinary ALA concentrations of 0.48 and 0.11 mg. per 100 ml., the CR concentrations of the urine specimens were 65 and 32 mg. per 100 ml. These samples were exceptionally dilute. It is not feasible, however, to set a minimum concentration for creatinine high enough to eliminate the other false negative, or the three borderline false negatives, all of which had CR concentrations above the mean.

The large standard deviation of CR concentrations and the direct relationship between CR and ALA concentrations led us to calculate the dimensionless value ALA-CR, defined as

$$\frac{[ALA]}{[CR]} \times 10^3$$

We anticipated that it might correct for ALA fluctuation caused by variation in urine concentration and thus offer improved correlation with blood lead observations, but this result was not

Figure 2. Urinary ALA concentration and urinary creatinine concentration

realized. The coefficient of correlation between blood lead concentration and ALA-CR was 0.30, the same as that between blood lead and ALA concentrations. There was a similar distribution of false positives and negatives. Reference to the blood lead value, within the limitations of our sample, does not confirm the validity of either the ALA or the ALA-CR scale.

Nor were the ALA and ALA-CR scales substantially consistent with each other. The coefficient of correlation between them was 0.40. Using this value to calculate the regression equation and the regression equation to predict a trace ALA-CR value corresponding to the minimum trace ALA concentration, we adopted the value of 6 as the minimum trace level on the ALA-CR scale.

The numerical value of this minimum is arbitrary and, at this stage, unimportant. Its usefulness lies in permitting the division of table 2 into four quadrants. Values in the two right quadrants are trace on the ALA scale. Values in the two upper quadrants are trace on the ALA-CR scale. The upper left and lower right quadrants, therefore, contain values for which the two scales disagree. Regardless of the exact positioning of the quadrant dividers, a substantial number of specimens remain in these areas of disagreement.

Discussion

Determination of urinary ALA on a large scale is feasible in terms of technical simplicity, acceptance by patients, and cost. It has potential value as a public health measure in alerting underprivileged communities to the dangers of lead poisoning and to the public agencies that can assist in improving health and housing standards. The practical potential of employing ghetto teenagers in large-scale screening to identify individual buildings where the danger of lead poisoning is particularly acute has been demonstrated.

The value of using ALA concentration in randomly collected urine specimens as a clinical screening test, however, remains problematical. Davis and associates (6), among others, have suggested that the inhibition of ALA dehydrase indicated by an increase in urinary excretion of ALA is a more sensitive and reliable index of the metabolic effects of lead than measurement of the blood lead level, since this level rises only when an established equilibrium distribution of lead between bone and soft tissue is disturbed (for example, by illness or renewed ingestion). Nevertheless we have demonstrated that, while increased concentration of urinary ALA follows a geographic and age distribution predicted by an association with lead ingestion, urinary ALA in a child's small specimen may not be elevated despite independent evidence of lead poisoning; also, elevation may occur in the absence of any corroborating evidence of plumbism.

We have further shown that the ALA concentrations found in the urine of young children correlate well with the concentrations of urinary creatinine. All of these results strongly suggest that, in the range encountered in a high-risk but asymptomatic population, variability in fluid balance may

Table 2. Urinary delta–aminolevulinic acid (ALA) and ALA-creatinine (CR) concentrations in 390 children

ALA-CR	Milligrams ALA per 100 milliliters urine													
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.3	2.5
19												1		
15														1
14					1									
13						1								
12									1					
11			1	1	1									
10		2		1		1						1		
9			3	1	1	2	1			1				
8			2	5	1	1		1	1	2				
7		4	3	8	6	4	1	1	2					
6	1	1	4	7	6	9	5			3	1			
5		2	15	7	10	8	5	1	2			1		
4		9	10	16	11	13	3	5	1	1	1		1	
3	1	17	17	27	16	9	3	1	1		1			
2	1	14	19	12	5	2	4	1						
1	3	8	3											
0		2												

play as important a role as metabolic changes in fixing the ALA concentration in small urine specimens collected under field conditions.

Collection of blood for simultaneous serum ALA and lead assay would help corroborate this hypothesis, and 24-hour collection of urine might help overcome the problem. Unfortunately, however, neither of these methods is readily adaptable to field screening. In the absence of a more reliable, equally simple technique, it would now seem worthwhile to apply the small sample ALA analysis to the first urine specimens voided in the morning or to the urine of children in relatively stable fluid balance, perhaps by avoiding collection of samples during hot weather.

Evaluation of any screening test for lead poisoning is handicapped by the absence of a definitive parameter of lead toxicity. Simple clinical evaluation is inadequate in detecting such long-term changes as behavior and learning disorders (8–11), nephropathy, and gout (12–14), which have been attributed to chronic overexposure to lead. Only a prospective study could convincingly demonstrate which of the many proposed standards of plumbism correlates well with these alleged sequelae. In view of the threat of massive yet subtle damage and the prospect that the project, once begun, will be painstakingly slow, a long-term prospective study of this kind cannot be undertaken too soon.

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Teenagers in a slum community carried out door-to-door screening in July and August 1968 in three areas of the Bronx, N.Y., for lead poisoning in children. Samples were collected from 390 children. Delta-aminolevulinic acid (ALA) concentration in small urine specimens was used as an index of increased exposure to lead. ALA levels among slum dwellers were significantly higher than among children in public housing; 16 percent of children in the slums, but none of those in public housing, showed concentrations in or above the trace range.

Correlation of urinary ALA concentration with blood lead concentration in 30 samples was only 0.30 ($P>0.10$), with notable false negatives, while correlation of ALA with urinary creatinine concentration in 390 samples was 0.55 ($P<0.01$), suggesting that this method is subject to significant error because of changes in the output of urine. Attempts to compensate mathematically for this error were unsuccessful.