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COMMENTARY

Screening techniques for undue lead exposure in children: Biological and practical considerations

THERE ARE, in the United States today, only two large-scale screening programs (Chicago[1] and New York City[2]) for the detection of undue exposure to lead, increased lead absorption, and lead poisoning in children. Data collected in these and earlier pilot studies[3-5] indicate that prospective screening of preschool-age children in old, deteriorated housing areas is likely to reveal that 5 to 10 per cent have unsuspected increased lead absorption and that 1 to 2 per cent may have findings compatible with the diagnosis of lead poisoning. A much larger number will show evidence of undue exposure to lead. The 5 year experience in Chicago is of particular interest because the data suggest that systematic screening in conjunction with an experienced central laboratory, community awareness, and an organized treatment and follow-up program have apparently significantly reduced the incidence of increased lead absorption and severe acute lead poisoning in the group of children being screened and followed. The prevalence of leaded paint in old housing[6-8] and the poor prospects for rapid abatement of this environmental hazard to children make it likely that children who live in old houses in many communities will have to be tested repeatedly during the preschool years. These considerations make the need for reliable and biologically sensitive, but simple, screening techniques abundantly clear. Several approaches which require 0.1 ml. of blood or less are now being field tested. Some measure lead in blood, whereas others are designed to measure one of the adverse responses to increasing concentrations of lead in the tissues.

Abbreviations: ALAD = δ-aminolevulinic acid dehydratase, FEP = free erythrocyte dehydratase, ALA = δ-aminolevulinic acid, CP = coproporphyrin, AAS = atomic absorption spectrophotometric, ASV = anodic stripping voltammetry, CaEDTA = edathamil calcium disodium.

The concentration of lead in whole blood may be the best index of the level of current and recent absorption of lead in children. The *risks* of acute adverse effects on health can also be estimated on the basis of blood

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lead concentration. In general, a continuum of increasingly severe, adverse metabolic, functional, and clinical effects may be correlated with several ranges of blood lead concentration. As described elsewhere,[9-11] four ranges may be recognized: (1) normal ($\overline{m} \cong 20$ μg Pb*; range 5 to 40 μg Pb),[12, 13] (2) undue exposure to lead (> 40 μg Pb), (3) increased lead absorption (50 to 80 μg Pb), and, (4) clearly evident clinical lead poisoning (usually associated with concentrations > 80 μg Pb). Concentrations greater than 40 μg Pb almost certainly indicate additional absorption of lead from a source(s) other than normal uncontaminated food and drink and, hence, undue exposure to lead. The biological adequacy of a screening technique may be judged by its ability to separate these four groups of children. Perhaps the most important criteria are a high degree of specificity for lead and sufficient sensitivity to identify children with blood lead concentrations in the 50 to 80 μg Pb range: Most children in this range may be expected to show evidence of impaired heme synthesis. Whether the subclinical thresholds for other significant adverse effects on the health of young children may also lie somewhere within this range is not now known.

The options for screening include measures of exposure and absorption (lead in blood, urine, and hair) and measures of some of the adverse metabolic responses to increasing concentrations of lead in the soft tissues (changes in osmotic resistance of circulating red blood cells, in vitro assay of δ-aminolevulinic acid dehydratase [ALAD] activity in peripheral blood, porphyrin in blood primarily free erythrocyte protoporphyrin [FEP]), δ-aminolevulinic acid (ALA) in serum, and ALA and coproporphyrin (CP) in urine. Elsewhere in this issue, Qazi and Madahar[14] report a promising and simple but relatively nonspecific screening technique based on alterations in osmotic resistance of red blood cells, and Specter and associates[15] present clear evidence of the inadequacy of the measurement of the concentration of ALA in random samples of urine as a screening technique in children. These and other options may be considered in terms of their biological sensitivity and specificity and their clinical and analytical practicality.

When the screening programs in Chicago and New York City were started, whole blood lead concentration was chosen as the most reliable direct index of exposure and the level of absorption and risk in the child. Methods of analysis requiring whole venous blood samples were selected despite the field sampling problems involved in securing a 5 ml. sample of blood; other methods of analysis available at that time did not yield results of sufficient accuracy. Other health departments which have provided a laboratory diagnostic service have long relied on blood lead determination because it is the single measurement which best fulfills the varied needs of health departments and physicians. Accurate determination of lead (and other trace metals) in body fluids requires scrupulous care in the collection of samples, as well as in laboratory procedures. Samples of blood and urine must be collected with lead-free equipment. (This equipment is often specially cleaned and supplied by the laboratory performing the analysis.) In the laboratory, reliable standard methods, quality control techniques, competent analysts, and continuous experience with the method are all essential. A recent interlaboratory study[16] revealed that only 40 per cent of the 61 participating laboratories reported results of acceptable precision for lead in blood. As a result of this study, Keppler and associates[16] made a number of recommendations including the following: adoption of standard methods for blood lead analysis, certification of laboratories, and the use and exchange of standard blood reference samples. The "USPHS" dithizone method[17] has served in recent years as a suitable reference method for other techniques. Criteria for the evaluation of the newer atomic absorption spectrophotometric (AAS) and other techniques may be found in the analytical literature[18, 19]: These techniques have the potential for far higher output than do pre-

*It is understood that blood lead concentrations are expressed throughout in terms of μg Pb per 100 Gm. of whole blood.

cise dithizone methods. Even so, the amounts of lead found in normal and slightly abnormal blood approach the detection limits and sensitivity of conventional atomic absorption spectrophotometric methods. Of particular interest with respect to the screening of children are several microtechniques which require 0.1 ml. of blood or less. Two are flameless AAS techniques[20-23] while a third AAS method[24] employs the flame. Although the initial reports indicate excellent agreement with conventional macro-AAS techniques, time and experience will determine whether other workers can obtain the same precision and accuracy in blood which the developers of each of these techniques have reported. Of particular interest is a highly specific electrochemical technique employing anodic stripping voltammetry (ASV).[25] This method, which is now being field tested, requires 0.05 to 0.1 ml. of blood for lead analysis; with this microsample, one need not work so close to the detection limit for lead as with the AAS method. It may be anticipated that one or more of these micromethods will prove suitable for clinical needs when more experience is gained in both field sampling and laboratory techniques. Past experience and other analytical considerations indicate that only those laboratories where lead analyses are made on a continuous basis are likely to produce reliable results: It is highly unlikely that a laboratory in which lead analyses are made sporadically can produce results of sufficient accuracy and precision.

Measurement of the concentration of lead in urine (μg Pb per liter) in casual samples of urine gives an insensitive and variable index of lead exposure; indeed, spontaneous urinary lead excretion may even be within normal limits in children with acute clinical lead poisoning.[26] This method has the poorest correlation with other indices of the "toxic" or mobile fraction of the total body lead burden. Aside from the difficulties of collecting urine samples from young children, it would be the poorest biological choice as a screening technique.

Measurement of lead in hair may prove to be a most useful epidemiologic tool for estimating differences in the levels of long-term community exposures to lead in varied groups of the population.[27] The washing technique for the removal of adsorbed air- and dust-borne lead from the hair is critical; even so, doubt still remains as to what portion of the remaining lead represents lead absorbed into the body, which is subsequently incorporated into the growing hair. Although lead in hair is of considerable interest to the epidemiologist, it does not now appear suited for the evaluation of individual human subjects.

Data in both children and adults are consistent with the hypothesis that arithmetic increases in blood lead concentration are associated with exponential increases in the mobile fraction of the total body lead burden as measured by the edathamil calcium disodium (CaEDTA) mobilization test and with exponential increases in the accumulation and excretion of heme precursors.[11] These relationships suggest that screening techniques based on measures of adverse responses, such as impairment in heme synthesis, may prove to be relatively insensitive to minimal increases in blood and tissue lead concentrations; on the other hand, they became progressively more sensitive (and toxicologically significant) at higher concentrations of lead in blood and soft tissues. The technique of Qazi and Madahar,[14] reported elsewhere in this issue, appears simple and adequate for screening purposes by the criterion of sensitivity, although the effect being measured has low specificity for lead. Normal children and those with blood lead concentrations greater than 60 μg were separated by their simple technique. Others[27, 28] who have studied the phenomenon of increased osmotic resistance of circulating red blood cells in occupationally exposed adults have noted that time, the concentration of sodium chloride in the incubating medium, and the increased mechanical fragility of red cells associated with plumbism may be critical factors in this test. Accordingly, those who attempt this screening approach would be well advised to adhere strictly to the microtechnique proposed by Qazi and Madahar.[14] As noted by these authors, the test is non-

specific so that confirmation by more specific measures and clinical evaluation is required. No "false negative" results are noted by them.

The most specific measure of response to increasing concentrations of lead in blood now known is the in vitro assay of ALAD activity in peripheral blood. Only acute alcoholism is known to cause a comparable effect.[30] Studies of venous blood samples from adults[31-33] and a few children[34] show a negative linear correlation between the log of ALAD activity and blood lead concentrations of 5 to 95 μg Pb and higher; all reports indicate that there is rather wide scatter about the regression line. Hernberg and associates[35] have recently reported serial observations in a few adults which suggest that in vitro ALAD activity may remain constant in a given individual with a constant level of exposure and a relatively constant blood lead concentration. A microadaptation of this procedure[36] showed little apparent capability to discriminate among children with blood lead concentrations between 40 and 120+ μg Pb, so that one half or more of the children tested by this technique would have to be retested by more sensitive alternate procedures. For example, the need for chelation therapy could not be based on such an insensitive test. Strande[37] reported that another micro-ALAD assay showed statistically significant correlation with blood lead concentration, but that the wide scatter made direct assay impractical as a screening technique. Perhaps when more is learned about the biological and technical aspects of the ALAD assay, microtechniques will be found which are suitable for clinical use in children. The concentration of ALA in serum is so low in normal persons that chemical determination is unlikely to be sufficiently sensitive for screening purposes; however, it is a useful measurement in persons with clinical acute lead poisoning.

Pilot studies[38, 39] indicate that the concentration of FEP in lead-poisoned children is 25 to 250 times greater than normal; biologically, this may be one of the most sensitive measures of adverse response to lead which has the potential for measurement in capillary samples of blood. Only erythropoietic protoporphyria, an uncommon genetic disorder, is associated with comparable elevations in FEP. Severe iron deficiency states[38] and diseases characterized by impaired utilization of iron may show lesser increments in FEP, so that measurement of FEP alone does not separate mild to moderately increased lead absorption from these disease states. However, no biological "false negatives" are likely to be found. The fluorescence of porphyrins makes them detectable in concentrations well below 0.1 μg per milliliter. Nelson and associates[39] used fluorescence microscopy to estimate porphyrin concentration semiquantitatively in fresh smears of peripheral blood. They report good correlation between the "fluorocyte count" and the response to CaEDTA in children with subclinical and clinical lead poisoning. Fluorescence microscopy for porphyrins has not found wide clinical use in the past because the technique apparently requires considerable experience and a technician with rapid color discrimination for the fleeting red fluorescence of protoporphyrin in blood smears. A sensitive microphotofluorimeter can bypass this human variable (color vision) and so facilitate the quantitative measurement of porphyrin in blood. This possibility is now being field tested.

Elsewhere in this issue, Specter and associates[15] report important negative data. They find that measurement of the concentration of ALA in random samples of urine from children is quite inadequate for screening purposes. This is in agreement with the report of Blanksma and associates[40] and with the findings of several unpublished studies which this commentator has seen. The problem is *not* technical; rather it is biological. Although quantitative daily urine collections yield meaningful data which are quite useful from the viewpoint of clinical research, the wide range of variation in the concentration of ALA and CP in individual urine samples makes this approach useless for screening purposes since subclinical increases in lead absorption cannot be differentiated from normal. Qualitative urinary tests for ALA and

CP do not clearly separate "normal from abnormal" until blood lead concentration exceeds the 80 to 100 μg range. The clinical usefulness of such urine tests in children[15, 41] is limited: These tests serve only as rapid emergency room diagnostic aids in ill children. In industry, these tests find wider use because urine can be collected under standardized conditions, serial observations can be obtained, and *trend* of serial observations can be interpreted together with other laboratory data and clinical evaluation.

For a number of reasons, the concentration of lead in blood is the single most useful index of exposure to lead and the *risk* of adverse effects associated with increasing concentrations of lead in the soft tissues. The fact that a number of laboratories do not at present perform this analysis with sufficient accuracy and precision does not negate the toxicologic importance of this measurement; rather, physicians and the Public Health Service should support local health agencies in their efforts to develop adequate laboratory capability for the needs of the community. Health departments, on which this task is most likely to fall, must have the capability to measure lead not only in body fluids but also in environmental samples. There is every reason to believe that one or more of the microtechniques for blood lead determination now in the developmental stage will be operational soon. When these methods are published, the thoughtful clinician will want to know the inherent analytic variation for each technique and how each compares with the established reliable methods which require the larger venous blood samples. The needs of physicians and clinics are somewhat different. Adequate evaluation, treatment, and follow-up of patients found to have increased lead absorption require measurements of both absorption and adverse response, together with careful clinical evaluation. Among the measures of response which have potential for screening are the techniques of Qazi and Madahar[14] reported in this issue, improved micro-ALAD assay and measurement of porphyrin in blood. It is most unlikely that any urine test will be suitable for the screening of young children. Techniques which do not give rise to false negative results, which reliably separate persons with blood lead concentrations greater than 50 μg Pb from those with normal values, and which can be repeatedly performed on capillary blood samples will provide considerable improvement over what has been available in the past. Ideally, a screening technique should also have the capability of identifying, as a separate group, those with blood lead concentration greater than 80 μg, since it is this latter group which is at greatest immediate risk.

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REFERENCES

1. Blanksma, L. A., Sachs, H. K., Murray, E. F., and O'Connell, M. J.: Incidence of high blood lead levels in Chicago children, Pediatrics **44:** 661, 1969.
2. Specter, M. J., and Guinee, V. F.: Epidemiology of lead poisoning in New York City—1970, presented at the American Public Health Association's Ninety-eighth Annual Meeting, Oct. 26, 1970, Houston, Texas.
3. Bradley, J. E., Powell, A. E., Niermann, W., McGrady, K. R., and Kaplan, E.: The incidence of abnormal blood levels of lead in a metropolitan pediatric clinic, J. PEDIATR. **49:** 1, 1956.
4. Griggs, R. C., Sunshine, I., Newill, V. A., Newton, B. W., Buchanan, S., and Rasch, C. A.: Environmental factors in childhood lead poisoning, J.A.M.A. **187:** 703, 1964.
5. Lin-Fu, J. S.: Lead poisoning in children. Social and Rehabilitation Service, U. S. Dept. of Health, Education, and Welfare. Children's Bureau Publication No. 452, Washington, D. C., 1967, U. S. Government Printing Office.
6. Schucker, G. W., Vail, E. H., Kelley, E. B., and Kaplan, E.: Prevention of lead paint poisoning among Baltimore children: A hard-sell program, Public Health Rep. **80:** 969, 1965.
7. Ingalls, T. H., Tiboni, E. A., and Werrin, M.: Lead poisoning in Philadelphia, 1955 to 1960, Arch. Environ. Health **3:** 575, 1961.
8. Barltrop, D., and Killala, N. J. P.: Factors influencing exposure of children to lead, Arch. Dis. Child. **44:** 476, 1969.
9. Chisolm, J. J., Jr.: Increased lead absorption—toxocological considerations. In press.
10. Statement approved by Surgeon General,

U.S.P.H.S.: Medical aspects of childhood lead poisoning. In press.
11. National Research Council, Washington, D. C.: Airborne lead in perspective. To be published.
12. Survey of lead in the atmosphere of three urban communities: Public Health Service Publication No. 999-AP-12, 1965.
13. Goldwater, L. J., and Hoover, A. W.: An international study of "normal" levels of lead in blood and urine, Arch. Environ. Health **15:** 60, 1967.
14. Qazi, Q. H., and Madahar, D. P.: A simple rapid test for lead poisoning, J. PEDIATR. **79:** 805, 1971.
15. Specter, M. J., Guinee, V. F., and Davidow, B.: The usefulness of random urinary delta aminolevulinic acid samples as a screening test for lead poisoning, J. PEDIATR. **79:** 799, 1971.
16. Keppler, J. F., Maxfield, M. E., Moss, W. D., Tietjen, G., and Linch, A. L.: Interlaboratory evaluation of the reliability of blood lead analyses, Am. Ind. Hyg. Assoc. J. **31:** 412, 1970.
17. Keenan, R. G., Byers, D. H., Salzman, B. E., and Hyslop, F. L.: The "USPHS" method for determining lead in air and in biological materials, Am. Ind. Hyg. Assoc. J. **24:** 481, 1963.
18. Zeegers, P. J. T., Smith, R., and Winefordner, J. D., Lewis, L. L., Pickett, E. E., and Koirtyohann, S. R.: *In* Beilby, A. L., editor: Modern classics in analytical chemistry, Washington, D. C., 1970, American Chemical Society.
19. Willis, J. B.: Atomic absorption spectrometry, *in* Mavrodineanu, R., editor: Analytical flame spectroscopy, New York, 1970, Springer-Verlag, pp. 525-598.
20. Matousek, J. P., and Stevens, B. J.: Biological applications of the carbon rod atomizer in atomic absorption spectroscopy. I. Preliminary studies on magnesium, iron, copper, lead, and zinc in blood and plasma, Clin. Chem. **17:** 363, 1971.
21. Amos, M. D., Bennett, P. A., Brodie, K. G., Lung, P. W. Y., and Matousek, J. P.: Carbon rod atomizer in atomic absorption and fluorescence spectroscopy and its clinical application, Anal. Chem. **43:** 211, 1971.
22. Donega, H. M., and Burgess, T. E.: Atomic absorption analysis by flameless atomization in a controlled atmosphere, Anal. Chem. **42:** 1521, 1970.
23. Hwang, J. Y., Ullucci, P. A., Smith, S. B., Jr., and Malenfant, A. L.: Microdetermination of lead in blood by a flameless atomic absorption spectrometry, Anal. Chem. **43:** 1319, 1971.
24. Delves, H. T.: A micro-sampling method for the rapid determination of lead in blood by atomic absorption spectrophotometry, Analyst (London) **95:** 431, 1970.
25. Matson, W. R., Griffin, R. M., and Schreiber, G. B.: Rapid sub-nanogram simultaneous analysis of Zn, Cd, Pb, Cu, Bi and Tl. Trace Substances in Environmental Health, Fourth Annual University of Missouri Conference, June 23-27, 1970, Columbia, Missouri, pp. 396-406.
26. Byers, R. K., Maloof, C. A., and Cushman, M.: Urinary excretion of lead in children. Diagnostic application, Am. J. Dis. Child. **87:** 548, 1954.
27. Hammer, D. I., Finklea, J. F., Hendricks, R. H., Shy, C. M., and Horton, R. J. M.: Hair trace metal levels and environmental exposure, Am. J. Epidemiol. **93:** 84, 1971.
28. Griggs, R. C.: Lead poisoning: Hematologic aspects, *in* Moore, C. V., and Brown, E. B., editors: Progress in haematology, vol. IV, New York, 1964, Grune & Stratton, Inc., pp. 117-137.
29. Hasan, J., and Hernberg, S.: Interactions of inorganic lead with human red blood cells. With special reference to membrane functions. A selective review supplemented by new observations, Work-Environment-Health **2:** 26, 1966.
30. Moore, M. R., Beattie, A. D., Thompson, G. G., and Goldberg, A.: Depression of δ-aminolaevulic acid dehydrase activity by ethanol in man and rat, Clin. Science **40:** 81, 1971.
31. Bonsignore, D., Calissano, P., and Cartasegna, C.: A simple method for determining delta-amino-levulinic-dehydratase in the blood, Med. Lav. **56:** 199, 1965.
32. Hernberg, S., Nikkanen, J., Mellin, G., and Lilius, H.: δ-aminolevulinic acid dehydrase as a measure of lead exposure, Arch. Environ. Health **21:** 140, 1970.
33. Nakao, K., Wada, O., and Yano, Y.: Delta-aminolevulinic acid dehydratase activity in erythrocytes for the evaluation of lead poisoning, Clin. Chim. Acta **19:** 319, 1968.
34. Millar, J. A., Cumming, R. L. C., Battistini, V., Carswell, F., and Goldberg, A.: Lead and δ-aminolaevulinic acid dehydratase levels in mentally retarded children and in lead-poisoned suckling rats, Lancet **2:** 695, 1970.
35. Hernberg, S., Nikkanen, J., Tola, S., Valkonen, S., and Nordman, C.-H.: Erythrocyte ALA dehydratase as a test of lead exposure. International Conference on Chemical Pollution and Human Ecology, Prague, October, 1970.
36. Weissberg, J. B., Lipschutz, F., and Oski, F. A.: δ-Aminolevulinic acid dehydratase activity in circulating blood cells: A sensitive laboratory test for the detection of childhood lead poisoning, N. Engl. J. Med. **284:** 565, 1971.
37. Strande, C. S.: A method for screening for lead exposure based on the assay of erythrocyte ALA dehydratase, Transactions American Pediatric Society, April-May, 1971, Atlantic City, N. J., p. 191, (Abst).
38. Watson, R. J., Decker, E., and Lichtman, H. C.: Hematologic studies of children with lead poisoning, Pediatrics **21:** 40, 1958.

39. Nelson, J. D., Dorn, P., Rogers, L. E., and Sartain, P.: Fluorescence of erythrocytes in relation to erythrocyte protoporphyrin and to urinary lead excretion, Am. J. Clin. Pathol. **50:** 297, 1968.
40. Blanksma, L. A., Sachs, H. K., Murray, E. F., and O'Connell, M. J.: Failure of the urinary delta-aminolevulinic acid test to detect pediatric lead poisoning, Am. J. Clin. Pathol. **53:** 956, 1970.
41. Benson, P. F., and Chisolm, J. J., Jr.: A reliable qualitative urine coproporphyrin test for lead intoxication in young children, J. PEDIATR. **56:** 759, 1960.