SCREENING FOR PEDIATRIC LEAD POISONING

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Prevention of injury to the developing nervous system in young children is dependent upon early detection of increased lead absorption during the preclinical stage of plumbism. Practical considerations limit the choice of screening tests in children to erythrocyte protoporphyrin (EP) and blood lead (Pb-B) which can be performed on micro samples of blood, which in turn, can be obtained by finger prick. In lead poisoning and in iron deficiency states, zinc protoporphyrin IX is the porphyrin found in peripheral erythrocytes. Results obtained with portable equipment and a simple field EP test are reported here for three groups of children. Simple EP tests serve as an inexpensive preliminary epidemiological tool to detect *groups* with increased lead absorption. This was demonstrated in relation to a stationary point source of exposure to lead: Preliminary screening with an EP test (which was later confirmed by extensive EP and Pb-B tests throughout the entire metropolitan area of El Paso, Texas, USA) indicated that increased lead absorption occurred in children residing in close proximity to the stationary point source, but not in those children residing far away. Dose-response (population response rate) data were determined prospectively in a well child clinic setting in an old housing area in Baltimore, Maryland in 155 preschool children. A 50 per cent positive response rate was found in those with Pb-B in the 40—49 μg percent range and a 95 percent positive response rate was found in those with Pb-B $\geq$ 50μg percent. Longitudinal trends in Pb-B and EP were determined in a cohort of 342 children who were tested at three month intervals for one year. Among these 342 children, 50.6 percent showed stable Pb-B, while 72.2 percent showed stable EP; i. e., the difference between the first and subsequent tests during the 12 month period did not exceed the 99 percent confidence limits for the methods of measurement. Overall, the trend in serial Pb-B tests was stable or could be explained clinically in 67 percent of the 342 children; similarly, trends in EP were stable or could be explained clinically in 91 percent of

these same 342 children. It was concluded that EP tests are suitable as preliminary screening tests in children and that it is the more stable biologic test for monitoring children, provided that the factor of nutritional iron status is taken into account. Moreover contamination is not a problem with micro EP tests.

With your indulgence, I shall direct my remarks today more specifically to the question of biologic monitoring for the prevention of pediatric lead poisoning. In particular, the long-range goal in young children is the prevention of permanent injury to the developing nervous system. For children, an effective screening or biological monitoring test should be reasonably simple and capable of being performed repeatedly in high-risk populations within the context of well child care on micro samples of blood obtainable by finger prick. For screening tests in children, a requirement for venous samples is not acceptable. It is, perhaps, worthwhile to contrast occupational and pediatric exposure to lead. In addition to the differences in age, there are other important differences which have a bearing on the approach to monitoring. Groups of workmen form a relatively closed community: The degree of exposure associated with various types of jobs is generally known and biological testing can be carried out systematically. Through medical surveillance and serial observation, variations in the response of each individual can be identified. By contrast, pediatric lead poisoning occurs in open, rather ill-defined populations and initial medical decisions often must be made on the basis of a single encounter. In the United States, the association between pediatric lead poisoning and old housing is clear. Ninety percent or more of pre-World War II housing is reported to have old lead pigment paints in areas accessible to young children (1, 2); yet, many if not most of the children living in such housing do not become ill. Clearly, other factors such as the state of repair of the various dwellings which the child may visit, parental stress and adequacy, variable degrees of pica in different children and variations in nutritional status play modifying roles. These modifying factors are not likely to be elicited during a single brief visit to the out-patient clinic. Past pediatric experience indicates that lead poisoning in young children is generally first recognized on the basis of central nervous system manifestations, if one depends on clinical signs and symptoms for diagnosis. Asymptomatic cases with increased lead absorption are usually found by examination of housemates of symptomatic index cases or by serendipity. It is also clear that chelation therapy begun *after* the onset of acute or »chronic« encephalopathy is not effective for the prevention of rather gross residual permanent brain damage in at least 25% of such cases. With repeated bouts of acute clinical illness, the frequency of residual injury increases. These consequences are potentially preventable through early detection, intervention and follow-up during the subclinical stage. Detection of the metabolic derangement in heme synthesis in the erythroid cells of the bone marrow caused by lead serves well for this preventive approach.

Derangement in heme synthesis is currently considered the critical or first adverse effect associated with increasing concentrations of lead in the soft tissues (3). The combination of decreased δ-aminolevulinic acid dehydratase (ALA-D) activity in red blood cells, increased urinary δ-aminolevulinic acid (ALA-U), increased urinary coproporphyrin (CP-U) and increased erythrocyte protoporphyrin (EP) is pathognomonic for lead, distinguishing it from all other disorders of pyrrole metabolism in man. The choice of screening tests currently lies among tests for blood lead (Pb-B) or one of these heme metabolites. In pediatric practice, urinary tests are limited to random samples. Urinary tests for ALA-U and CP-U have proven unreliable in young children, primarily because of the wide variation in their concentration in urine (4) in random samples and difficulties in getting toddlers to void on request. The practical options narrow down to tests for Pb-B and erythrocyte protoporphyrin (EP). Both can be performed on micro samples. Both are stable in blood so that samples are transportable without refrigeration. Micro EP tests are simple to perform. Unfortunately, samples for ALA-D require considerable care in handling, refrigeration and prompt measurement. On practical grounds, this limits the usefulness of ALA-D for screening. At least 95% of the porphyrin in whole blood is protoporphyrin IX bound to red blood cells. Simplified micro fluorometric tests which do not, in fact, separate the various porphyrins, are for practical purposes »specific« for protoporphyrin because the occurrence of increased amounts of other porphyrins in human blood is quite rare. Three conditions are associated with elevated erythrocyte protoporphyrin: 1) lead poisoning, 2) iron deficiency and 3) one of the uncommon inborn errors of porphyrin metabolism; namely, protoporphyria (synonyms: erythropoietic protoporphyria, erythrohepatic protoporphyria). In protoporphyria, protoporphyrin IX is present in circulating red blood cells as the free base (5). In plumbism and iron deficiency, the major fraction in the circulating red blood cells is the metalloporphyrin, zinc-protoporphyrin. These different forms can be distinguished by their fluorescence spectra. Standard methods for extracting protoporphyrin from blood involve extractions with ethyl acetate-acetic acid mixtures, followed by reextraction of porphyrins into hydrochloric acid. In this process, zinc-protoporphyrin is hydrolyzed and included in the total protoporphyrin extractable under these conditions which is measured as protoporphyrin IX dication. This, by custom, is called »free« erythrocyte protoporphyrin or »FEP«. Techniques for measuring FEP require more steps and take at least twice as long to perform as simpler tests involving measurement of zinc-protoporphyrin. Furthermore, they call for trained laboratory personnel, whereas simpler procedures can be taught to persons without laboratory training. Further technical aspects of these tests will be discussed later.

I now wish to report some screening experience over the past four years with a simple one-step fluorometric protoporphyrin assay adapted for field use. The method (6) and some clinical trials with it have already been reported (7, 8) The method involves partial extraction and hydro-

lysis of zinc-protoporphyrin so that samples may read at 635 nm against protoporphyrin IX standards. Values found are about one-seventh of those found by standard FEP micro methods. In potassium-EDTA anticoagulated blood, the normal value in children with hematocrit $\geq 36\%$ and <30 µg Pb/100 ml blood is 4.2 ± 1.96 µg/100 ml blood expressed as protoporphyrin IX; In heparinized blood, it is somewhat lower: 3.17 ± 1.36 µg/100 ml blood. The upper limit of normal (mean ± 2 S. D.) is 8 µg PROTO/100 ml blood. This is also the value which on the basis of regression analyses predicts 40 ± 10 µg Pb/100 ml blood and is used as the cut-off point to separate normal from non-normal (7). Briefly, one takes into the field 7x75 mm tubes prefilled with acidified acetone (600 µl acetone containing ing 44.7 µg of 0.01 MHCl/liter). At the test site, 44.7 µl blood is dispensed into the tube. The mixture is vortexed for 30 seconds, centrifuged for 1 minute and read in a fluorometer which is set to constant sensitivity with coproporphyrin standards.

Today I will report results for two groups of children with dissimilar sources of exposure to lead. Group I is composed of 130 children ranging in age from four months to 15 years who were prospectively screened in El Paso, Texas in March, 1972, in relation to lead exposure from a stationary point source of lead emissions from a large smelter. *Landrigan* and coworkers (9) have recently reported the results of a very comprehensive epidemiologic study in children drawn from the entire metropolitan El Paso area and *Mc Neil* (10) will report later in this symposium on detailed studies in the children of Smeltertown who lived closest to the smelter. I will not dwell on these points, but wish to point out how, armed with a portable fluorometer and a roadmap obtained in a local petrol station, we were able to define the localization of increased lead absorption in children within 48 hours, on the basis of the protoporphyrin results. Venous blood was drawn for Pb-B, but the analyses of several hundred samples were not completed until about two months later. Please also bear in mind that in March of 1972, there was wide-spread speculation in El Paso that perhaps all young children throughout the metropolitan area were absorbing excessive amounts of lead emitted by the smelter. Disparate preliminary Pb-B data had only added to this speculation. Figure 1 shows a map of the El Paso area. The smelter and Smeltertown 200 meters from the base of the main stack and slightly downhill and downwind from the stack and slag piles are designated in this figure. Also note the location of Old Fort Bliss. The main metropolitan area of El Paso lies over the mountains and to the east of the smelter, Smeltertown and Old Fort Bliss. Of the 130 children tested, 26 lived in Smeltertown. Fifty-seven children lived in the Old Fort Bliss apartments which were 2.5 km downwind from the stack. The remaining 47 children were drawn from elsewhere and lived either upwind or greater than 2.6 km from the smelter. Figure 2 shows the percentage of children with elevated screening protoporphyrin values (≥ 8 µg) and the percentage with Pb-B ≥ 40 µg Pb/100 ml. They are divided into three groups according to residence in Smeltertown, Old Fort Bliss or other

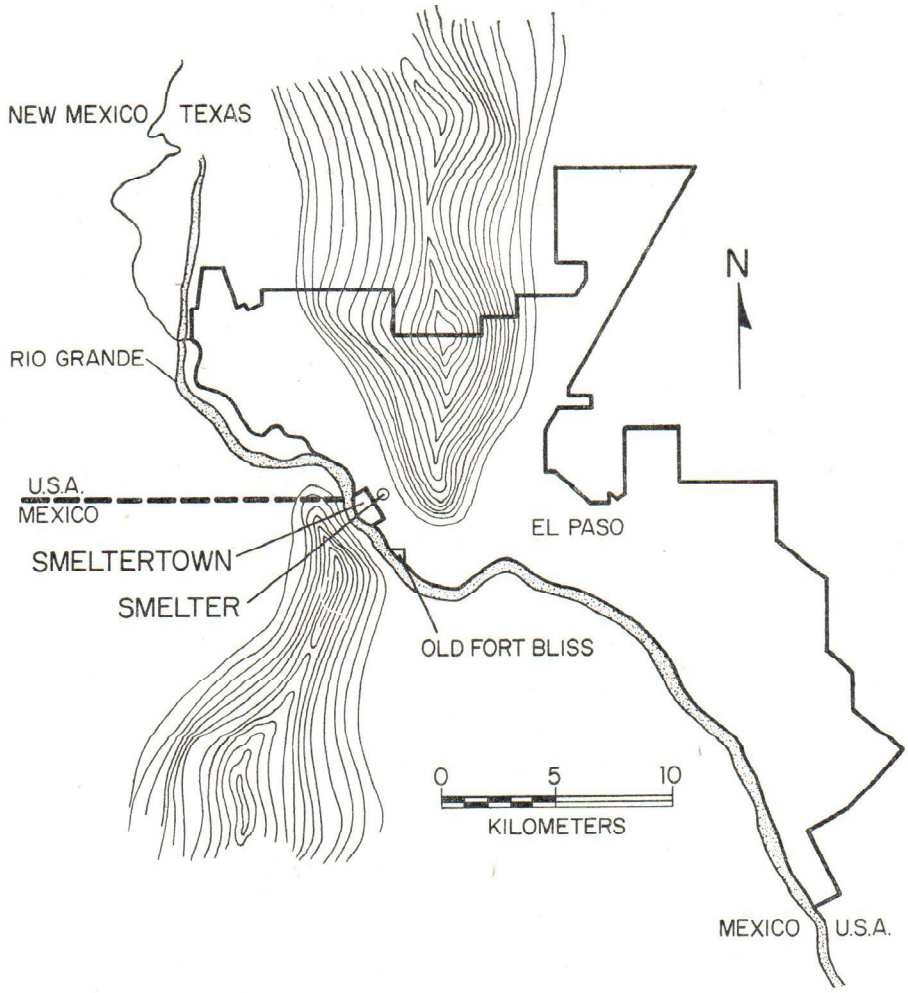

Fig. 1. *A map of the El Paso area*

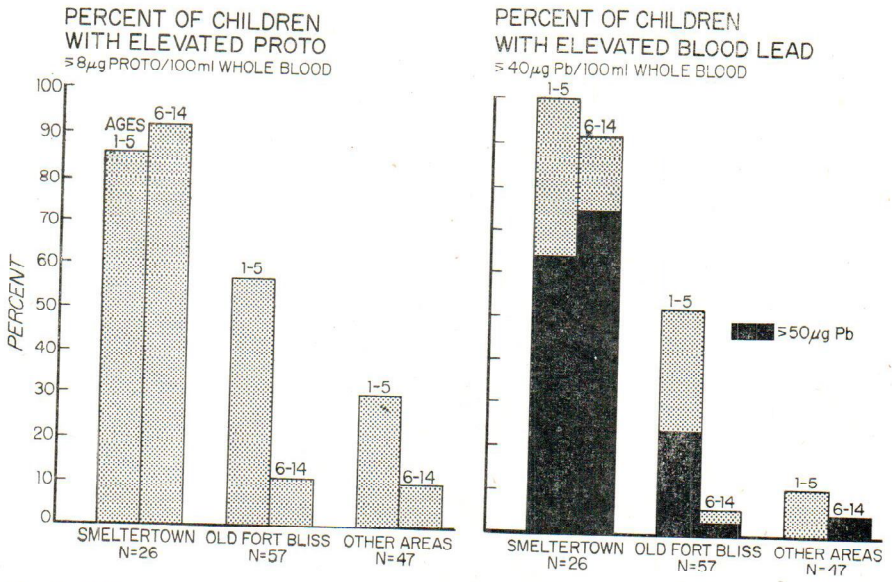

Fig. 2. *The percentage of children with elevated screening protoporphyrin values and percentage of children with elevated blood lead*

areas. It was immediately clear at the time of testing that 90% of the 26 children from Smeltertown showed elevated protoporphyrin. In other areas, the percentage of children six to 14 years of age with abnormal protoporphyrin results was low ($\leq$ 10%) and the frequency of elevated protoporphyrin in the one to five year age group was much less than in Smeltertown. Thus, we concluded tentatively within 48 hours from the fluorometric assays that increased lead absorption probably involved almost all of the Smeltertown children, that some pre-school children in Old Fort Bliss were affected but that there was probably no significant public health problem elsewhere in the metropolitan area. When the Pb-B values shown on the right hand of Fig. 2 were available some two months later, these preliminary observations were confirmed. Later, very extensive Pb-B testing confirmed this preliminary estimate (9). This study is presented as an example of the utility of simple protoporphyrin assays as an inexpensive preliminary epidemiological tool to detect *groups* with increased lead absorption.

Next I wish to report on current experience in Baltimore, Maryland in an on-going screening program under the auspices of the John F. Kennedy Institute. In this program, simultaneous micro blood samples for analysis of EP, Pb-B and hematocrit are being obtained by fingerprick according to the micro Pb-B sampling and analysis technique developed by *Mitchell* and coworkers of the New York State Health Dept. (11, 12,

13) Duplicate 50 μl aliquots were analyzed for Pb-B by *Mitchell* and coworkers. The standard deviation for this method is $\pm 3\mu$g Pb/100 ml blood. Duplicates differing by > 9 μg Pb/100 ml blood are rejected as unsatisfactory or contaminated. On this basis, approximately 2% of 2500 samples have been rejected during the past 18 months. Protoporphyrin was measured by the one-step screening technique immediately in duplicate 44.7 μl aliquots of blood. Micro hematocrits were also measured.

Before presenting the screening results, a word concerning the mode of presentation may be in order. In evaluating a screening test, it is desirable when possible to test it prospectively against a valid diagnostic test for the disease in question (14). In this way, the sensitivity and specificity of the test can be determined and subjects may be identified as properly classified, false negative or false positive (15, 16). Most of the screening tests proposed during the past decade for plumbism have been judged on this basis against Pb-B on the tacit assumption that it is the true diagnostic test of disease, despite the fact that it is the measurement of the concentration of the metal in blood at a particular point in time and not a measurement of the effect of lead, and hence, not a direct indicator of diseases or impaired metabolism or function. Clearly, Pb-B does reflect exposure or what *Zielhuis* (17) has termed the external dose of lead. However, there is as yet no substantial body of evidence in humans that Pb-B does, in fact, reflect the internal dose of lead at its site of action in the tissues (3). Erythrocyte protoporphyrin, on the other hand, is one of the metabolic indicators of lead's effect in the erythroid cells of the bone marrow (3, 17). An alternate method of looking at the relationship between blood lead and erythrocyte protoporphyrin concentrations is in terms of the dose-response concept of toxicology. This concept, recognizing the uniqueness of the individual, states that the rate of positive response in a population (percent showing a given effect) will increase in proportion to a quantitative increase in the dose of a toxic substance, but that there will be both highly susceptible and highly resistant individuals who may be classified as »reactors« and »non-reactors« at each dose level until 100% response is reached. The typical dose-response curve is presented by an »S« shaped curve with dose plotted on the abscissa (x) and response rate plotted on the ordinate (y). Figure 3 shows the theoretical form of this curve. Figure 4 shows the dose-response curve for the protoporphyrin effect as determined prospectively in 155 young children between June and November of 1974. The curve is plotted from the initial test results. Tests were carried out primarily in two well child care clinics which serve an old inner city pre-World War II housing area. Age distribution is as follows: < 1 year $= 1$, 1 yr $= 62$, 2 yr $= 24$, 3 yr $= 28$, 4 yr $= 22$ and ≥ 5 yr $= 18$. The main source of exposure is old flaking lead-based paint in the homes. Note first that the response rate does not reach zero and that 8.8% of this group with Pb-B < 30 μg Pb/100 ml show some deviations from normal. This is due to background effect (18) which in this case is attributable to iron deficiency, which is also rather common in such children. Note

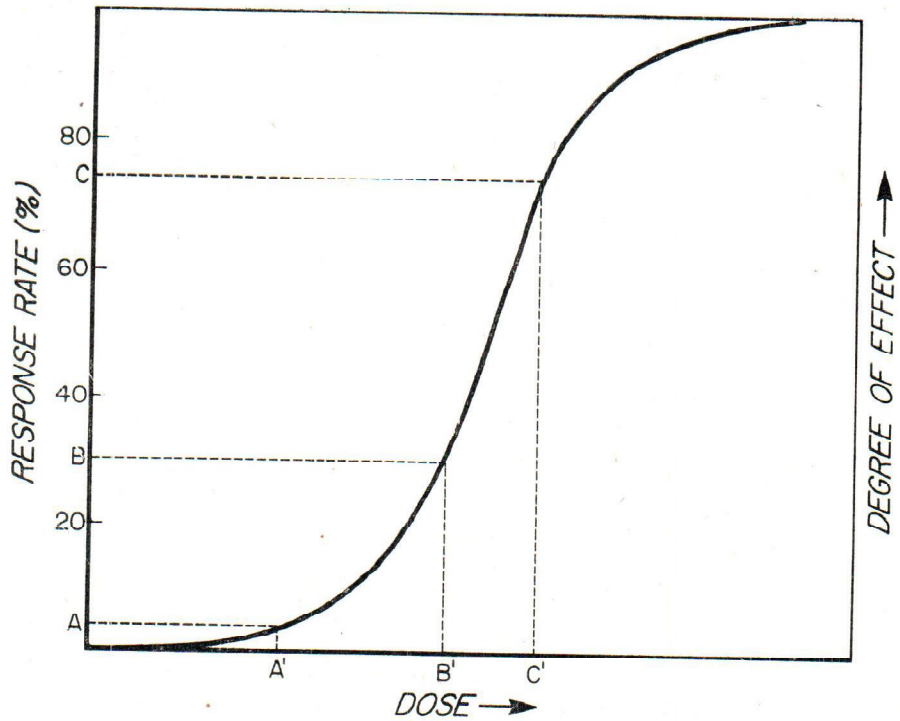

Fig. 3. *The typical theoretical dose-response curve*

also that 95% or more of those with Pb-B $\geq$ 50 μg Pb/100 ml show a positive response. Looked upon from the dose-response viewpoint, published micro tests for FEP suggest the presence of a similar pattern (19). In this particular population, 18% show both Pb-B $\geq$ 50μg Pb/100 ml and elevated protoporphyrin, while 20% may be classified as entirely normal. The 50% response rate is associated with the 40—49 μg Pb/100 ml range in Pb-B. Although not shown, the degree of effect can be graded according to the degree of elevation in erythrocyte protoporphyrin. Simultaneous micro hematocrit measurements provide an indicator of the presence of anemia.

It is the monitoring of trends of absorption and effect that are important in preventive pediatric management. Table 1 summarizes experience in this inner city population in 342 pre-school children who have been periodically tested at approximately three month intervals during the past year with simultaneous micro Pb-B and erythrocyte protoporphyrin tests. In this summary table, serial Pb-B values which do not vary by more than 9 μg Pb/100 ml from the initial test are classified as stable or »no change« and the erythrocyte protoporphyrin test results are

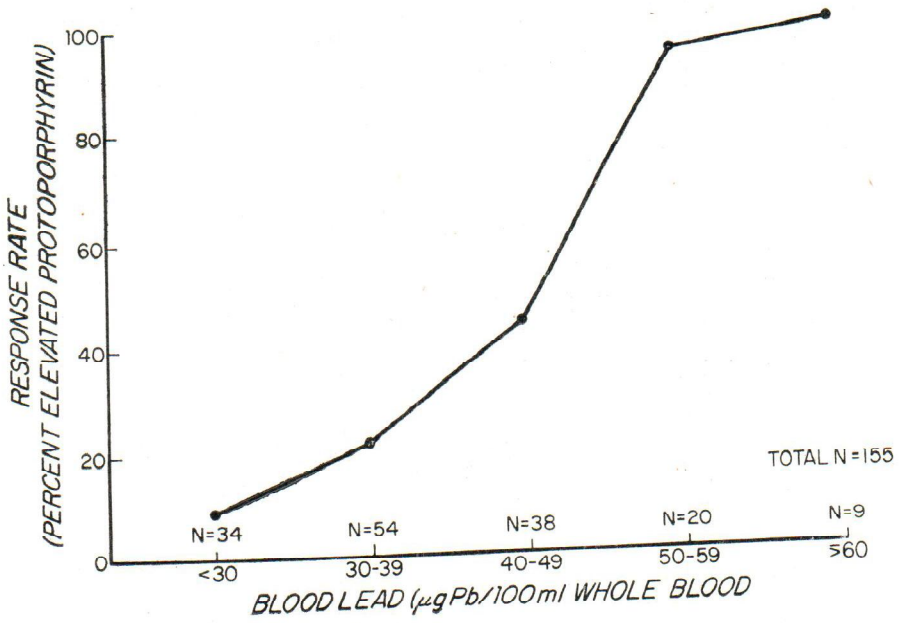

Fig. 4. *Dose-response curve for the protoporphyrin effect as determined prospectively in 155 young children*

Table 1

*Trends in the concentrations of lead and erythrocyte protoporphyrin in blood**

	Blood lead concentrations	Erythrocyte protoporphyrin concentrations
No change (Beyond analytical variation)	173 (50.6%)	247 (72.2%)
Change in trends** (Greater than expected analytical variation)	169 (49.4%)	95 (27.8%)
Total	342	342

* Tests for Pb-B and EP repeated at approximately 3 month intervals. Trends observed in 342 children initially tested between August 1973 and May 1974 at the John F. Kennedy Institute, Baltimore, Maryland

** 99% confidence limits for methods of analysis:
Pb-B ± 9 μg/100 ml
EP ± 4 μg/100 ml

classified as stable if variation between tests is $\leq$ 4 μg. These limits were selected on the basis that they represent the 99% confidence limits for the methods of measurement. On this basis, 50.6% of the group show stable serial Pb-B values and 72.2% show stable serial EP tests. Table 2 summarizes an evaluation of those in whom the change

Table 2

Trends in the concentrations of lead and erythrocyte protoporphyrin in blood (explanation of cases in which changes occurred)

Cause of change	Blood lead concentrations	Erythrocyte protoporphyrin concentrations
Chelation therapy	26	26
Iron therapy	9	15
Change in exposure*	12	12
Consistent one-directional Change**	8	11
Unknown	114	31
Total	169	95

* Both tests change in the same direction due to 1) presence of child in the home during burning and scraping for removal of old paint, or 2) child moved into public housing which does not contain lead paint.

** Consistent change in one test, but not the other; undocumented change in exposure is suspected.

in either Pb-B or erythrocyte protoporphyrin exceeded the limits of analytical variability as defined above. In 26, the change in both parameters can be attributed to chelation therapy. In 12 children, changes in both tests were clearly attributable to a change in exposure, either through change in residence or the presence of the child in the home during the burning and scraping of paint. However, in 68% or 114 of 169 instances of unstable serial blood lead values, there was no clear explanation. In the case of changing serial erythrocyte protoporphyrin tests, there was no ready explanation for 33% or 31 of the 95 unstable cases. Overall, the trend in serial blood lead tests was stable or could be explained in 67% of the cases; similarly, trends in erythrocyte protoporphyrin tests were stable or could be explained in 91% of the 342 children. For this study, one of the best available collection and analysis systems for blood lead was deliberately selected. Even so, erythrocyte protoporphyrin tests appear to provide a more stable biological monitoring test.

During this time, federally-funded programs were restricted to micro blood lead tests: No other type of screening test was then permitted. Elsewhere, in Baltimore, a different system of collection and analysis was being used. During this time, at least 25 children known to me were hospitalized and a number treated with chelating agents in whom follow-up blood lead tests was appreciated, erythrocyte protoporphyrin tests later, were found to be normal or borderline. When this wide fluctuation in blood lead tests was appreciated, erythrocyte protoporphyrin tests were requested by some physicians prior to any treatment. Informal conversations with pediatricians elsewhere indicate that testing of children on the basis of blood lead only has led to similar unsatisfactory experiences in the attempted management of individual cases. In recognition of the many problems associated with micro blood lead tests and their interpretation in individual children, the Center for Disease Control (DHEW) in March 1975 published new guidelines for screening and management in children (20). In this statement, four risk categories based on both blood lead and erythrocyte protoporphyrin are recognized: normal, borderline, moderately elevated and highly elevated. In terms of blood lead, these groupings correspond approximately to the following ranges: < 30, 30—49, 50—79 and $\geqq 80$ μg Pb/100 ml blood. However, under these guidelines, when the erythrocyte protoporphyrin test places the child in a different category from that indicated by simultaneous blood lead measurement, the erythrocyte protoporphyrin value is to take precedence in determining the classification of the individual case.

Serial observations in three children illustrate the responsiveness of erythrocyte protoporphyrin tests to changes in exposure, the influence of chelation therapy and presumed redistribution of lead within their tissues during the early follow-up period. In Figure 5 the numbers indicate blood lead values at various points in time. Serial erythrocyte protoporphyrin values for each child are connected by lines. The dotted portion of each line signifies no treatment, while the solid lines show the trend during chelation therapy. At the time of the initial test, it can be seen that blood lead values were in the 50 to 60 μg range for all children, but that two of the children showed substantially higher protoporphyrin values. During the first month of follow-up, the children were at home. Also at this time, there was a death in one of the adult members of the family which possibly diverted the mother's attention. Also, the burning and scraping of paint began. One month later, blood lead and erythrocyte protoporphyrin values had clearly risen in all three children and they were hospitalized and placed on chelation therapy. Note at the end of the first treatment course that blood lead values are virtually identical in all three cases, yet two show protoporphyrin values which are still elevated, while the third child's value is close to the upper limit of normal. That child received no further therapy and her test values remained stable for the next ten months. During this hospitalization, repairs within the home were completed. Even so, the two older children showed rebound in both protoporphyrin and blood lead. Treatment with

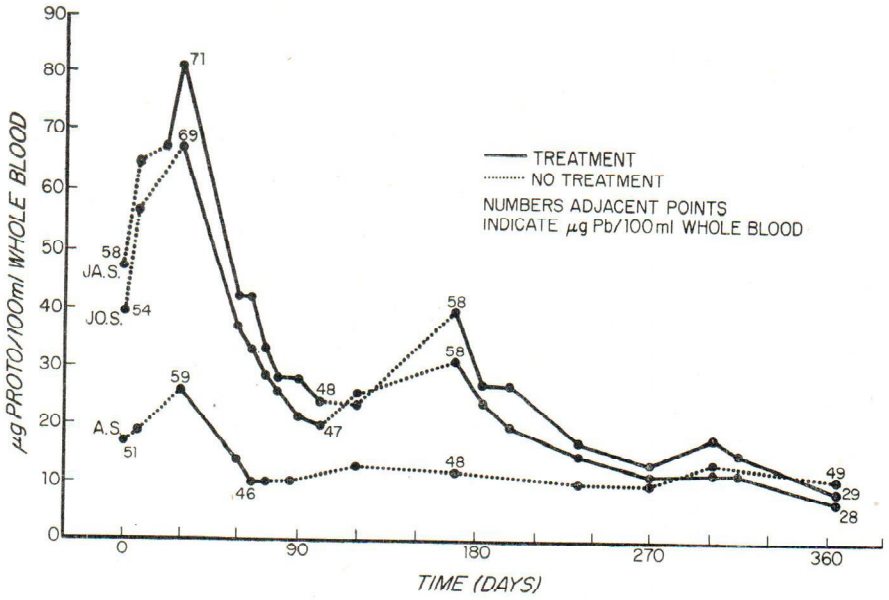

Fig. 5. *The influence of chelation therapy on erythrocyte protoporphyrin values in three children. The solid lines show the trend during chelation therapy while the doted portion of each line signifies no treatment. The numbers indicate blood lead values at various points in time*

d-penicillamine was recommenced with corresponding subsidence in values. These two children differ from A. S. in that they had positive bone films indicating increased boney storage of lead. The period of follow-up actually extends for an additional year, during which one child received a third course of d-penicillamine on the basis of rebounding protoporphyrin and blood lead values. This demonstrates the sensitivity of serial erythrocyte protoporphyrin tests in monitoring long-term responses to chelation therapy: Such measurements are especially useful during d-penicillamine therapy, since this drug removes lead from red blood cells thereby producing an artificially low Pb-B value. This effect of d-penicillamine on Pb-B limits the usefulness of Pb-B measurements during treatment. Micro zinc-propotorphyrin measurements also appear quite sensitive to acute changes in the dosage of chelating agents.

Figure 6 shows the fluorescence spectra found in the HCl-acetone extracts of blood used in these screening studies. Note that maximal excitation of the 635 nm emission peak is at 408 nm, while maximal excitation of the 595 nm emission peak is at 415 nm. Although zinc-protoporphyrin does have two emission peaks at approximately 595 and 635 nm, the ratio found in these extracts is not comparable to the ratio for

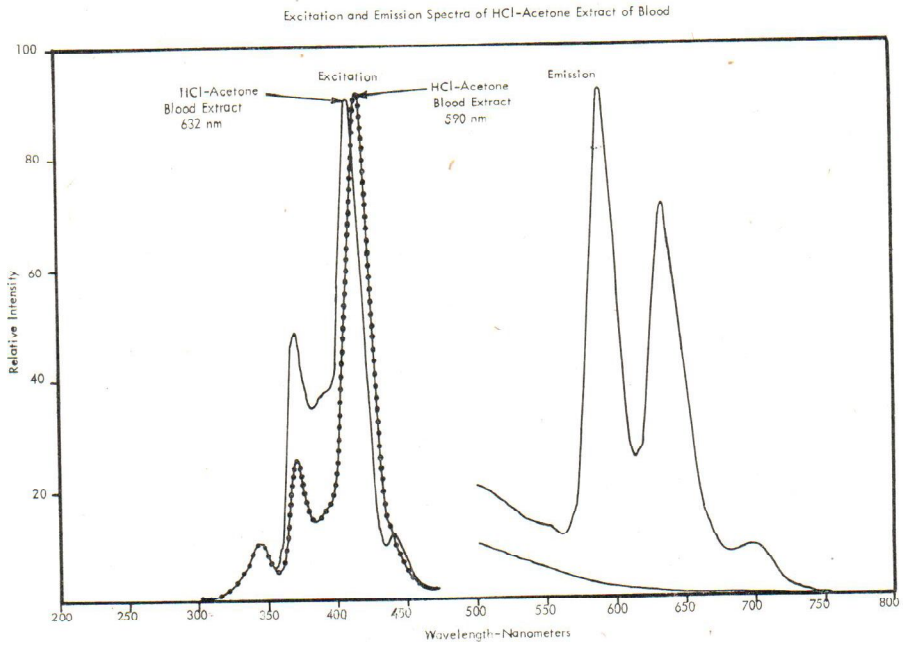

Fig. 6. *Excitation and emission spectra of HCl-acetone extract of blood*

pure zinc-protoporphyrin. The fluorescence data indicate the presence of two species in the HCl-acetone extract which is interpreted as a mixture of zinc-protoporphyrin and free protoporphyrin. Fortunately, the extraction technique gives constant values from day-to-day in individual children (7). Recently, we have found that extraction of blood with acetone containing acetic acid, instead of HCl, extracts zinc-protoporphyrin without hydrolysis of this metalloporphyrin. Figure 7 shows fluorescence spectra of extracts of blood with this acetic acid-acetone solvent in comparison with spectra for pure zinc-protoporphyrin in the same solvent system. Emission and excitation spectra are virtually identical. Our preliminary data suggests that 70 to 75% of protoporphyrin IX extractable by classical procedures is, in fact, the metalloporphyrin zinc-protoporphyrin IX. We have been stimulated to pursue this work because it offers a simple single-step test, as well as an opportunity to study zinc-protoporphyrin-FEP ratios under various conditions. Furthermore, with the Bowman-Aminco spectrophotofluorometer equipped with an R-446 photodetector available to us, we have been unable except at very high concentration, to measure zinc-protoporphyrin reproducibly directly in diluted blood, as proposed by *Lamola* and coworkers (5) who used a more sensitive spectrophotofluorometer.

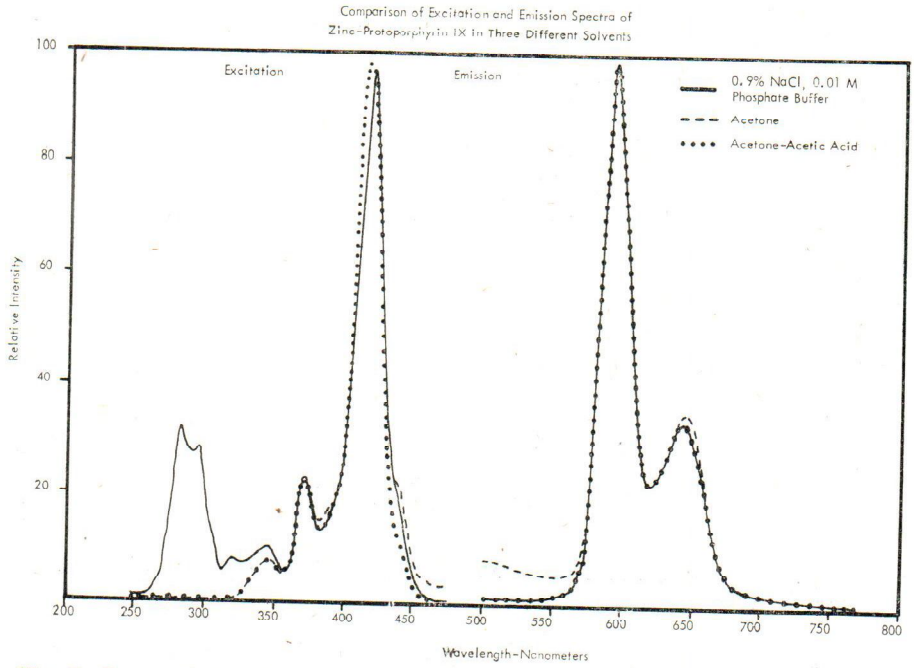

Fig. 7. *Comparison of excitation and emission spectra of zinc-protoporphyrin IX in three different solvents*

There have been important technical advances in microfluorometry for erythrocyte protoporphyrin recently. Perhaps, the most important is the availability within the past six months of stable zinc-protoporphyrin standards.* These greatly simplified the standardization of both zinc-protoporphyrin and FEP measurements. The Bell Laboratories group (5) have also developed a prototype portable fluorometer for measuring zinc-protoporphyrin directly in a drop of blood, using front optics and reflectance fluorometry. The instrumental requirements for FEP measurements are discussed elsewhere (21). Most important, FEP in EDTA-anticoagulated blood is stable for up to eight weeks and can be measured with a precision of ± 5%. Even so, use of a common millimolar absorption coefficient for protoporphyrin IX would facilitate interlaboratory comparisons. Instruments must be individually calibrated. The measurement of micro FEP can also be automated (22). In the interpretation of FEP and zinc-protoporphyrin, the possibility of coexisting iron deficiency must always be taken into account.

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In closing, I would like to raise some questions. Several studies concerning the possible adverse effects of subclinically increased lead absorption on the developing nervous system in young children have recently been reported. Results have been conflicting. In almost all of these studies, groupings are based on blood lead. Is it possible that Pb-B under non-steady state clinical conditions is not a reliable indicator of the concentration of lead at the site of action in the target cells of the bone marrow, nervous system and kidney? Does this biological factor, together with the analytical limitations in Pb-B measurement contribute to the uncertainties in these conflicting studies? In an attempt to evaluate this question, we have made simultaneous measurements of ALA-U, EP, Pb-U, Pb-B and chelatable lead in a small group of children with Pb-B in the range of 48 to 68 μg Pb/dl blood (23).

Intercomparisons suggest significant linear dose-effect relationships for chelatable lead and Pb-U against ALA-U and EP, but not for Pb-B against ALA-U and EP. These and other data (3, 4, 23) suggest that Pb-B may be the least appropriate indicator of internal lead dose against which to judge the adverse effects of lead. It further suggests the need for simultaneous use of an indicator of effect such as erythrocyte protoporphyrin in the biological monitoring of human populations who are at increased risk for plumbism. Finally, the validity of using heme metabolites such as erythrocyte protoporphyrin in preventive monitoring is based in part on the assumption that derangement of heme synthesis in the erythroid cells of the bone marrow is, in fact, the first or critical adverse effect (3) of increased lead absorption. However, no biochemical

Table 3

Relationship between indicators of dose and indicators of effect

Indicators of effect (dependent variables)		Indicators of dose (Independent variables)			
		Blood lead*	Urinary lead**	Chelatable lead***	
				1st 24 hr	3-day total
δ-aminolevulinic acid in 24 hr urine sample	N =	10	10	10	10
	r =	0.371	0.879	0.912	0.913
	p =	>.1	<.001	<.001	<.001
Erythrocyte Protoporphyrin	N =	10	10	10	10
	r =	0.649	0.700	0.746	0.785
	p =	<.05	<.05	<.02	<.01

* Range of blood lead levels = 48—66 μg Pb/100 ml whole blood

** Range of urinary lead levels = 32—129 μg Pb/24 hr

*** Range of chelatable lead = 0.72—5.89 μ mol Pb excreted/m mol CaEDTA administered

tests of comparable sensitivity for the detection of impairment in nervous system function due to lead are presently available for human studies so that, for the present, there remains an uncertainty about the identity of the first or critical adverse effect of lead. Currently, interesting experimental studies on the neurochemical effect of excessive lead are in their early stages (24, 25). These may shed light on this question.

ACKNOWLEDGEMENT

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Sažetak

KONTROLA OTROVANJA OLOVOM U DJECE

Prevencija oštećenja centralnog nervnog sistema sastoji se u prvom redu od rane detekcije prekomjerne apsorpcije olova još prije nastupa ikakvih kliničkih simptoma. S obzirom na praktične razloge, masovne analize ranih biokemijskih lezija teško su izvedive pa se u ovim slučajevima pretrage ograničavaju na mjerenje količine protoporfirina u eritrocitima i olova u krvi. Za tu se svrhu možemo koristiti mikrometodama i dovoljan je uzorak krvi iz jagodice prsta. Mjerenje protoporfirina prilagođeno je čak i za terenske uvjete i unatoč tome što sadrži niz slabosti s obzirom na specifičnost, vrijedno je sredstvo za screening velikog broja uzoraka.

U istraživanje su uzete tri skupine djece s različitim stupnjevima ekspozicije olovu. Ukupno je bilo 130 djece između 4 mjeseca i 15 godina, koja su živjela u blizini velike topionice u El Pasu u Teksasu. S obzirom na lokalitet, mogle su se odvojiti tri skupine. Od 26 djece iz neposredne blizine topionice, 90% ih je pokazivalo povećanu količinu protoporfirina (> 8 μg/100 ml). U drugim je skupinama djece, koja su živjela podalje od topionice, postotak povećane količine protoporfirina bio znatno manji. Ovi su rezultati bili poznati već 2 dana nakon uzimanja uzoraka a naknadno su bili u potpunosti potvrđeni rezultatima mjerenja koncentracije olova u krvi iste djece.

U drugom istraživačkom programu vrednovala se metoda određivanja protoporfirina u krvi 342 gradske djece i uspoređivala s vrijednostima olova u iste djece. Uzorci su uzimani iz jagodice prsta i olovo je određivano paralelno u dva laboratorija. Rezultati koji su se razlikovali za više od 9 μg/100 ml krvi odbacivali su se, pa je tako odbačeno 2% od 2500 rezultata. Koncentracija olova u djece tijekom jednogodišnjeg razdoblja nije se mijenjala u otprilike 50% djece, dok je koncentracija protoporfirina u eritrocitima ostala nepromijenjena u 72% djece. Razlog promjenama u najvećem je postotku bio nepoznat. U manjem je postotku bio uzrokovan terapijom kelirajućim sredstvima ili je pak ekspozicija olovu bila promijenjena. Prema tome trend promjene koncentracije protoporfirina u eritrocitima bio je lakše objašnjiv nego trend promjene koncentracije olova.

Iz svih se ovih pokusa dâ zaključiti da je određivanje koncentracije protoporfirina u eritrocitima dobar pokazatelj stupnja izloženosti djece i ima određene prednosti pred mjerenjem koncentracije olova u krvi u prvom redu zbog brzine analize i odsutnosti problema kontaminacije mikrouzoraka.

DISCUSSION FOLLOWING THE PAPER

ZIELHUIS: 1. In a non published human volunteer study in males, PbB 40 ± 5 μg/100 ml, we found evidence that FEP in erythrocytes gave a more adequate indication of dosage and of »internal exposure« than PbB. Do you agree that particularly in non steady state exposure FEP should be measured in addition to PbB, to predict *true* internal (tissue) exposure?

2. We have evidence (experimental, epidemiological) that adult women and children have a lower no-effect level of PbB in regard to increase of FEP and that increase of FEP with increasing PbB is steeper in females (and children) than in males. Had you any experience on this apparently *increased susceptibility* of *females* (and children) in comparison to males?

CHISOLM: Yes, we do have preliminary evidence that there is a statistically significant relationship between FEP and ALA-U (mg/M² (body surface area)/24. hrs.) and the »internal dose« of lead as measured by a standardized EDTA mobilization test for lead. In the same patients with Pb-B in a rather narrow range (48—68 μg Pb/100 ml whole blood), no significant relationship between Pb-B and ALA-U was found. Therefore, in the usual »non-steady state« situation in human populations, FEP would be preferrable to Pb-B in these circumstances.

I do not have any comparisons at present by age and sex concerning the no-effect level for FEP. I would not expect a sharply defined threshold based on previous experience with ALA-U. I would further comment that we have detected a few instances of unsuspected exposure to lead in testing adults to establish normal values for FEP. Elevated FEP values were found in women removing old paint by burning and scraping in the course of remodeling their homes. Other instances were related to iron deficiency. In young children, we find that iron deficiency contributes significantly to the number of elevated FEP values found in children with Pb-B in the range of 30—40 μg Pb/100 ml whole blood.

KOSTIAL: Our data on lead toxicity in rats indicate exactly the opposite. Males seem to be more sensitive to lead poisoning than females — according to our LD_{50} values after a single intraperitoneal application of lead acetate. In young animals which have a higher lead retention in the body and various organs lead toxicity also seems to be lower than in males. Could not this indicate that males might be more sensitive to other deleterious actions of lead which affect survival, irrelevant of the lead effect on heme synthesis?

CHISOLM: The Baltimore City Health Department has mortality data for lead poisoning dating from 1931. Their records show no difference between male and female deaths or acute encephalopathy in young children primarily between 1 and 3 years of age. I am not aware of sex-related differences in the very young reported by others. Whether the nervous system in the very young is more or less sensitive than the hematopoietic system is unknown. However, the experimental work of Carson and coworker and Silbergold and Goldberg, Goyer and others strongly suggests that the very young are more sensitive to lead than mature animals.

ZAVON: We cannot view the student iron values as normal for the population. In some work done in Cincinnati during the Miami Valley Project, we studied the diets of students and found them to be greatly deficient in many instances and in such a way that it is possible that the iron concentrations could be affected.

ZIELHUIS: 1. In our experiment [see E. J. Stuik, Int. Arch. Arbeitsmed. 33 (1974) 83] we compared male with female students. There is no reason to assume a more deficient diet in female students than in males.

2. Our findings were confirmed in an epidemiological study in Belgium [H. A. Roels *et al.*, Int. Arch. Arbeitsmed., 33 (1974) 277]: in female workers FEP rose much more steeply with increasing PbB levels than in male workers.
3. Up till now Fe metabolism has not been studied in that respect; the possible explanation that relative Fe-deficiency in females might be the underlying factor, does not appear unreasonable, but it still is a hypothesis to be studied.

ALESSIO: 4 years ago I read a paper of yours in which you studied the levels of serum ALA in children. I would like to know if this test is useful for screening studies. In fact in the industry we frequently use the determination of ALA in urine and unfortunately the levels of ALA sometimes show very high variation in the same subjects when we use urinary spot samples. Thank you.

CHISOLM: The method of serum ALA to which you refer was designed for the study of children with acute encephalopathy in whom 2—3 ml of plasma is needed. For a person with near normal plasma ALA one needs > 10 ml plasma; therefore, the method is not really intended or suitable for work with asymptomatic persons. We are now working on techniques to micronize the procedure. This is possible and should make it suitable as a screening method. It will still *not* be as simple as erythrocyte protoporphyrin methods.

BERLIN: To what extent the stability tests of changes of 4 μg/100 ml for Zn protoporphyrin and 9 μg/100 ml blood are comparable?
The Zn protoporphyrin screening test seems to be quite sensitive for screening individuals with blood lead levels above 40 μg/100 ml. Can this test be used at lower blood lead concentrations?

CHISOLM: An increase in Pb-B from 20μg to 80 μg Pb/100 ml whole blood represents a four-fold increase and is biologically significant. In the screening test for erythrocyte protoporphyrin which we used in these studies, the comparable increase is from 4 to 25 μg/100 ml whole blood or a six-fold change. This makes this particular screening test for EP somewhat more sensitive. In fact, in the analysis of the data I have biased the study against erythrocyte protoporphyrin test. With a newer Zn protoporphyrin test, which we are now developing, sensitivity and precision will be better. Both Zn protoporphyrin and FEP tests may prove sensitive to blood lead concentrations < 40 μg/100 ml whole blood. However, these tests are also quite sensitive to latent iron deficiency. To minimize the influence of iron status we have looked at children with hematocrits $> 36\%$ separately. In these »non-anemic« children we find no regression of Pb-B against erythrocyte protoporphyrin at blood lead concentrations < 35 μg Pb/100 ml whole blood.