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Threshold for lead damage to heme synthesis in urban children

(erythrocyte protoporphyrin/environmental health/ferrochelatase)

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ABSTRACT **Although lead has no physiological function and is present in only negligible amounts in the blood of remote populations, it has become customary to accept the usual blood Pb level(s) (BPb) observed in industrialized society as "normal." Pb interferes with many biochemical systems, among them the heme biosynthetic pathway; this is reflected by an exponential increase in erythrocyte protoporphyrin concentration (EP) as BPb increases. The present study estimated the threshold BPb at which an increase of EP occurs in a population of urban children. In the 2,004 children studied, BPbs ranged from 2 to 98 μg/dl, with 1,852 having a BPb of ≤30 μg/dl, a value presently considered normal. Preliminary analysis suggested that an exponential increase in the concentration of EP occurred after a threshold BPb (apparently between 12 and 20 μg/dl) was reached. Precise definition of the threshold BPb for an increase of EP was next determined by two approaches: segmented line techniques and probit analysis. Whether the entire population was analyzed or only the subset of samples with "normal" BPb (≤30 μg/dl), both methods yielded a threshold BPb of 15–18 μg/dl (average value, 16.5). These studies indicate that the heme synthetic pathway is affected by Pb at a level of exposure commonly observed in urban children, which is well below the limit that is presently too easily accepted as normal.**

Lead is a nonessential element to the human body. However, accumulation of excessive amounts in the human body is an unavoidable hazard of living in our present day environment (1). There is a gradient in the body burden of Pb [expressed by the blood Pb level (BPb)] from the nearly negligible values observed in remote populations (2, 3) to the highest levels observed in urban dwellers (4). Urban children, who are exposed to high concentrations of Pb in dust from vehicular emission and often from Pb-containing paint in deteriorated housing, tend to have the highest BPb among nonindustrially exposed populations (4). High doses of Pb in children result in severe neurological toxicity, leading to death in the most severe cases (5); exposure to lower doses results in more subtle damage (6). Because Pb is a poison of the SH groups and has affinity for several intracellular structures, it exerts its damage by interference with essential enzymes and cellular functions (7). Among the biochemical pathways that are damaged by Pb, the process of heme synthesis stands prominent because of its physiological importance to all tissues. In view of our detailed knowledge of this pathway, of the availability of sensitive techniques for its study, and of the ease of sampling blood, damage caused to it by Pb can be precisely quantitated. Fluorometric techniques allow precise measurements of the erythrocyte protoporphyrin concentration (EP) (8). This is the substrate of the last step of heme synthesis (i.e., the insertion of iron into the completed protoporphyrin molecule), which takes place in the mitochondria and is catalyzed by the enzyme ferrochelatase, located in the inner cristae. EP increases exponentially with BPb (9), reflecting in the peripheral blood the interference by Pb on mitochondrial function in the erythroid precursors in the bone marrow and in all other tissues.

Previous studies of the effect of Pb on EP have focused on individuals, both urban children (9–11) and adult Pb workers (12, 13), with BPb clearly increased above the usual range. The present study was directed to estimate the threshold BPb for an increase of EP in a population of urban children, of which the great majority had BPbs below the value which is currently called "normal" (14).

MATERIALS AND METHODS

Population Sampled. The experimental sample consisted of 3,830 venous blood specimens obtained in 1976 from children at a variety of locations throughout New York City, collected in Pb-free heparinized tubes, and submitted for Pb analysis to the New York City Department of Health Bureau of Laboratories. Each sample was accompanied by a form that supplied the following information: name, address, birth date, sex, ethnic group, and whether the child had been pretested, hospitalized, or suspected of Pb poisoning or whether the sample was taken for the purpose of screening. In addition, the computerized records of the New York City Bureau of Lead Poisoning Control, where every child found to have a BPb of >40 μg/dl has been listed since 1973, were analyzed. A review indicated that 2,597 of these samples had been obtained exclusively for primary screening for Pb poisoning. The remaining 1,233 samples were excluded for one of the following reasons: duplicate sample, pretested child, suspicion of Pb poisoning, chelation therapy, BPb >40 μg/dl at any previous time, hospitalization for any reason, or incomplete data. The samples were next sorted by age, and an additional 593, obtained from children age <24 months, were also excluded to minimize the frequency of EP increase secondary to iron deficiency (FeD), which is prevalent in this age group (15). The final study group consisted of 2,004 blood samples from children ages 2–12 yr (mean age, 5.5 yr; median age, 4.7 yr), obtained exclusively for primary screening for Pb poisoning from ambulatory children. However, the records of all discarded samples were kept in a separate file to verify whether their inclusion would significantly alter the later findings.

BPb and EP Measurements. BPb was measured by atomic absorption spectrophotometry (16). EP was measured by ex-

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Abbreviations: BPb, blood lead level; EP, erythrocyte protoporphyrin concentration; FeD, iron deficiency; AmLev, *d*-aminolevulinic acid; df, degrees of freedom.

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traction and fluorometry (8). Both BPb and EP were measured in μg/dl of blood; hereafter, these units will be omitted. The laboratory that performed the analysis participates successfully in the proficiency testing program of the Center for Disease Control and the New York State Department of Health for both tests.

RESULTS

BPb. In the 2,004 samples in the study group, BPb ranged between 2 and 98, with a distribution that approached log normality. The geometric mean, $\bar{x}$, was 17.3 ($\log_{10} \bar{x} = 1.238 \pm 0.227$).

Correlation Between BPb and EP. EP increased exponentially ($\log_{10}$ EP = 1.099 + 0.016 BPb; correlation coefficient, $r = 0.509$; degrees of freedom (df) = 2,002; $P < 0.001$). In a sample of this size, the existence of correlation, even at this level of significance, does not necessarily imply a relationship between the dependent variable (EP) and the independent one (BPb) that is constant throughout the entire range of the latter. The effect may occur only in the higher range; yet, when the entire group is analyzed as a whole, the predominance of samples in the higher range gives the impression of a significant correlation throughout. Therefore, we analyzed two close, but nonconsecutive, subsets of samples—one with the lowest BPb (2–12) and the other with slightly higher levels (20–30). It became apparent that, in the group with the lowest BPb, there was no correlation with EP ($r = -0.021$; df = 313; $P = 0.71$), whereas, in the group with the higher BPb, there was a significant exponential correlation ($\log_{10}$ EP = 0.923 + 0.021 BPb; $r = 0.254$; df = 634; $P = 0.003$). When all samples with BPbs of >20 were considered, an essentially similar significant correlation persisted ($\log_{10}$ EP = 0.910 + 0.022 BPb; $r = 0.603$; df = 784; $P < 0.001$). [It must be noted that both of these slopes of correlation are essentially the same as in our original study, which had included 1,038 children, 647 of whom had BPbs of >30 (9).] These findings suggested that the exponential increase of EP with BPb does not occur until the latter reaches a given value but thereafter continues at a steady rate, without evidence of a plateau phenomenon, at least in the range of our sample. Thus, a preliminary analysis indicated the existence of a threshold BPb (presumably between 12 and 20) above which EP increases at a constant exponential rate. The further detailed analysis was then directed to achieve a precise definition of this threshold BPb by appropriate techniques.

Estimates of the Threshold BPb for EP from the Entire Population (2,004 Samples). *Segmented line techniques.* The data were analyzed by the segmented curve-fitting technique of Hudson (17), which estimates the intersection (join point in the terminology of refs. 17 and 18) of two regression lines. This has been modified by Hasselblad *et al.* (18) to determine a "hockey stick" regression, specifically to measure threshold levels of biological effect. These two techniques use identical methods to estimate the intersection (threshold level); the Hasselblad technique assumes a horizontal line (no effect) below the threshold level, an assumption probably more valid in biological situations. The intersection was estimated at 17.7 (confidence limits = 16.2 and 21.5) by the Hudson technique and at 18.3 (confidence limits = 17.1 and 20.2) by the Hasselblad technique (Fig. 1*A*).

Probit analysis. The increase in EP with BPb indicates a dose–response relationship to exposure to Pb because a linear increase in BPb reflects an exponential increase in Pb body burden (7). This relationship can be measured best by the classical technique of probit analysis (19), requiring evaluation of the frequency of an event in groups of individuals exposed to progressively increasing doses. Thus, the data were subdivided into 13 categories: the first category included BPbs 2–10, the next 10 categories spanned the BPbs between 11 and 30 (2 μg/

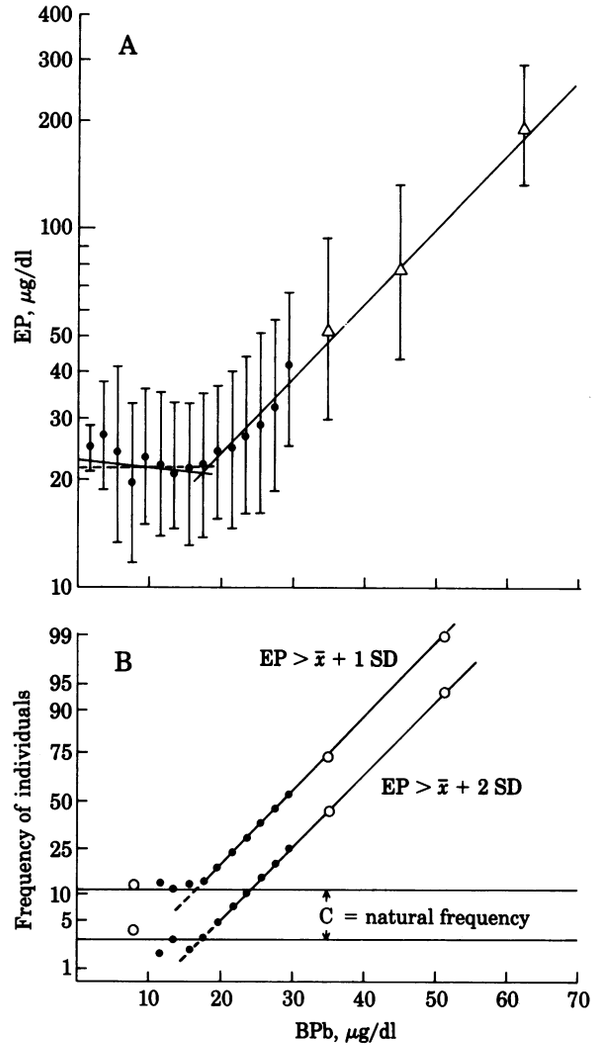

FIG. 1. Determination of the threshold BPb for EP increase with the entire population sample. (*A*) By segmented line techniques. The threshold level was determined from the intersection of two exponential regression lines of EP on BPb. By the method of Hudson (17), the threshold level of 17.7 was estimated from the intersection of the two solid regression lines. The method of Hasselblad *et al.* (18) assumes a horizontal (no effect) line (shown as a dashed line), which intersects the regression line of positive slope identical to that derived above, at a threshold level of 18.3. All 2,004 data pairs were used in these regression analyses. For graphic representation, the data have been grouped on the basis of BPb as follows: ●, 1–30 (15 pairs); and △, 31–40 (mean = 34.7), 41–50 (mean = 44.8), and 51–98 (mean = 62.7). The vertical bars from each point indicate one SD from the group mean. (*B*) By probit analysis. The data were grouped into 13 categories on the basis of BPb as follows: ●, 11–30 (10 pairs); and ○, 2–10 (mean = 8.1), 31–40 (mean = 34.7), and 41–98 (mean = 51.1). From the lowest BPb group, a "normal" reference EP was determined to be 21.7. For each BPb category, the probit of the frequency of individuals with EPs of >33 and >53 [the geometric mean $\bar{x}$ plus 1 SD (F1) and the mean plus 2 SD (F2) of the reference category, respectively] was plotted against the corresponding BPb. By iterative technique, the ordinate value *C* (representing the "natural" occurrence of the effect) was determined to be 10.7% for F1 and 2.4% for F2. By using only BPb 17–98, the probit regression lines for F1 and F2 were derived. The intersection of these regression lines with their respective ordinate value *C* occurred at BPbs of 16.4 and 16.6, respectively. These values indicate the threshold at which response exceeds the "natural" frequency.

dl each), and the final groups included BPbs 31–40 and 41–98, respectively. Within each category, EP distributions approached log normality, suggesting that any progressive increase with BPb is due to an effect on the entire population and not just on a few more-sensitive individuals. In view of the known effect of Pb on EP, also shown by the data, it is difficult to establish a normal reference group because, in New York City, children with BPb in the range expected in humans living in an uncontaminated environment (2, 3) are extremely rare. Therefore, the lowest BPb category (2–10) was chosen as the most appropriate reference group. This consisted of 131 samples with a geometric mean EP of 21.7 ($\log_{10} \bar{x} = 1.336 \pm 0.198$). For each of the 13 BPb categories, the frequency of individuals with EPs of >33 and >53 (the mean plus 1 SD and plus 2 SD, respectively, of the reference category) were measured and were called F1 and F2, respectively. When the values were transformed into provisional probits and plotted, it became obvious that, at low BPb, the frequencies were rather low but constant and then increased at higher BPb in a linear fashion.

These findings suggested a plateau of no response followed by a dose-dependent response. In this situation the intersection of the probit regression line with the plateau represents the threshold of the dose–response effect. Probit analysis allows a precise estimate of the plateau, the ordinate value *C*, which corresponds to the natural occurrence of the effect in the population. (In this case, *C* reflects the frequency of EP increase due to non-Pb-related causes, such as FeD or other minor chronic diseases.) The values of *C* were determined by iterative probit analysis (19) with all 13 BPb categories. These were 10.7% for F1 and 2.4% for F2. Next, probit regression lines were computed by using *C* set at 0 and only the highest BPb categories. A series of such regression lines was computed by utilizing initially only the seven BPb groups from 40–98 down to 21–22 and then adding into the computation the next lower group; the intersection of each line with the ordinate value *C* was then computed (we shall refer to this as an intercept with the C ordinate). As progressively lower BPb groups were added, there was no significant decrease in the values of the intercept until the BPb group 15–16 was included. Thus, the lines derived with the nine BPb groups between 17 and 98 were taken to provide the most correct intercept values. Both these probit regression lines intercepted the *C* ordinate near the abscissa value of 16.5 (16.4 and 16.6, respectively; Fig. 1*B*). For both lines, the observed and expected frequencies were not significantly different ($\chi^2 = 3.337$, df = 7, $P > 0.8$; and $\chi^2 = 3.539$, df = 7, $P > 0.8$, respectively). The intercept values indicate the dose level at which the response starts to exceed the natural frequency (threshold level of the effect). Thus, these data indicate that when the BPb exceeds the value of 16, the frequency of individuals with elevated EP increases in a dose-dependent relationship.

Estimates of the Threshold BPb for EP from the Normal Population of Children (1,852/2,004 Samples). Because a BPb of 30 is the currently accepted upper limit of normal (14), further analysis was then limited to the 1,852 pairs of values from children with BPbs of ≤ 30. Confining the analysis only to this group provides a more accurate estimate of the threshold BPb at which an increase of EP starts in children who would not require medical attention under current standards of care. Additionally, removing from the analysis children with BPbs clearly in the abnormal range avoids any possibility that a more pronounced effect of Pb in the higher range may result in deviation from the linearity of the regression line, which, in turn, may influence the estimation of the intersection.

Segmented line techniques. By using the techniques of segmented line analysis, the intersection was estimated at 15.4 (confidence limits = 12.9 and 18.2) by the Hudson method and at 16.5 (confidence limits = 14.3 and 18.5) by the Hasselblad method.

Probit analysis. Both probit regression lines intercepted the *C* ordinate near the abscissa of 16 (15.9 and 16.4, respectively). Again, for both probit regression lines, the observed and expected frequencies were not significantly different ($\chi^2 = 2.380$, $P > 0.7$, df = 5; $\chi^2 = 2.048$, $P > 0.8$, df = 5).

The threshold estimates obtained by utilizing only the data from children with BPbs in the range presently considered normal appear to be in close agreement with those obtained by using the entire group. Therefore, the previous estimates cannot be ascribed to an artifact.

DISCUSSION

The clinically adverse effects of exposure to large Pb doses are well established (4). It appears logical that there should be a progression from negligible effects at minimal dose, through mild effects at moderate dose, to serious clinical effects at very large dose. However, mild effects are often difficult to document in single individuals. Because Pb is a nonessential element and an extremely toxic one, Patterson postulated in 1965, on the basis of purely geophysical considerations, that the Pb content of the natural man (i.e., man unexposed to the environmental redistribution of Pb caused by human activities) should be negligible (1). Recent studies have clearly shown that, at extremely low exposure, the body Pb content is negligible. Ancient human skeletons have a Pb content several orders of magnitude lower than that in modern bones (20, 21). Even today, the BPbs of remote populations [both "acculturated" (3) and "unacculturated" (2)] are much lower than those of populations living in the industrialized world, with values approaching the low levels predicted by Patterson (1). These studies reaffirm the concept that the Pb body content observed in populations from industrialized countries reflects environmental pollution; therefore, their BPbs as currently observed cannot be considered normal because they are so much higher than in the natural man.

Patterson also predicted that adverse biological effects of Pb would become obvious when searched for with appropriate techniques. This hypothesis also is supported by recent experimental evidence. In 1970, Hernberg and co-workers demonstrated that the activity of the enzyme *d*-aminolevulinic acid (AmLev) dehydratase is inversely proportional to BPb (22). The rate of inhibition is exponential without any apparent threshold; 50% inhibition occurs at a BPb as low as 16.7 (a value typical for most urban dwellers and well within the range presently called normal), and inhibition of 90% occurs at a BPb of 55.5, above which overt clinical symptoms become frequent in children (4). Removal of Pb from the enzyme by means of SH-group reagents results in its complete reactivation (23). The exponential decrease in AmLev dehydratase with an increase in BPb is reflected by an exponential increase in urinary excretion of AmLev (24). The accumulation of EP in severe Pb poisoning has been known for decades (25). The introduction of sensitive fluorometric techniques by our laboratory led to the demonstration that the EP increases exponentially with a linear increase in BPb (8, 9). These observations were confirmed both in children (10, 11) and in adult Pb workers (12, 13). Most of these studies have utilized populations with excessive exposure to Pb, including our initial report that was based on a large number of children, the majority of whom had BPbs of >30 (9). It generally has been assumed that no threshold BPb exists. Recently Roels and coworkers compared two small groups of rural children with different BPbs because only one lived in the proximity of a smelter, and they suggested a lack of correlation with

the EP in the group with the lowest BPb (26). However, because there was no overlap in BPbs between the two populations in their studies, an estimate of the threshold level is not possible from their data. The population of children screened in New York City offered, on the other hand, an unusual opportunity for this type of evaluation because it included an adequately large number of individuals, the majority of whom had BPbs within the "normal" range, and its overall BPb distribution was log normal as expected (4). The analysis of the relationship between BPb and EP confirmed the exponential correlation. However, a preliminary analysis suggested that this may not be present throughout the entire range. The data were then analyzed with techniques appropriate to measure threshold levels: (*i*) the segmented line techniques, which estimate the threshold as the intersection of two regression lines, and (*ii*) the probit analysis, which yields a threshold at the intersection between the slope of the dose–response relationship and the line of natural frequency of the dose-unrelated effect. The analysis was first performed with all data, including 1,852 samples with BPbs of ≤30 (within the so-called normal range) and 152 with BPbs of >30. The segmented line techniques estimated the threshold at 17.7 by the method of Hudson and at 18.3 by the Hasselblad modification; the probit analysis estimated the threshold at 16.4 and 16.6. Next, the analysis was repeated with only those 1,852/2,004 samples with BPbs of ≤30. The restriction of the analysis to this group was motivated by the desire to assess the threshold level in that segment of the population that had BPbs certainly within the "normal" range. The segmented line techniques estimated the threshold at 15.4 by the method of Hudson and at 16.5 by the Hasselblad modification. The probit analysis estimated the thresholds at 15.9 and 16.4. Regardless of the technique used and of the inclusion or exclusion of samples from children with abnormal BPb, all of these estimates are, in the worst case, within 3 μg/dl of each other (range, 15.4–18.3). These findings clearly indicate that the threshold BPb for an increase of EP in urban children occurs between 15 and 18, with an average estimated value of 16.5. (A value of 16 may be the most valid because a range of 15.4–16.5 was obtained when the analysis was restricted only to those samples with "normal" BPbs.)

Probit analysis also estimates the dose at which 50% of the population responds. The natural occurrence of EP increases in children due to non-Pb-related causes (*C*) can be removed by using the Abbott formula (27) to compute the BPb at which 50% of the children show an effect exclusively from Pb exposure. By using this technique, the data were reevaluated. The BPbs at which 50% of the children had increased EP exclusively from Pb exposure were computed to be 29.9 and 35.2 for values that were >1 and >2 SDs above the mean of the reference group. (Corresponding values without the Abbott correction are similar—28.6 and 35.6, respectively.) An EP of >50 in a child with a BPb of >30 is presently considered an indicator of excessive exposure to Pb requiring further medical attention (14). Our study indicates that, at a BPb of 30, 27% of the children will have an EP of >53 (exceeding by 2 SD the mean of the reference group). Thus, even at the cutoff point of normal BPb, a substantial percentage of the population exhibits evidence of Pb-induced biochemical damage.

Increase of EP indicates an impairment of the completion of the synthesis of heme, a process that culminates with the insertion of iron at the center of the protoporphyrin molecule. Increased EP may result not only from Pb intoxication but also from a variety of other causes, such as chronic infections, certain hemolytic anemias, and FeD. The latter is the most frequent in children. FeD aggravates Pb intoxication both by increasing Pb absorption (28) and by enhancing the effects of Pb on heme synthesis. On the other hand, Pb intoxication leads to FeD by reducing iron absorption (29). These interreactions are, at least in part, mediated by the existence of a common carrier in the intestinal mucosa for both metals (30). In urban children, both FeD and Pb intoxication tend to occur with the greatest frequency in the same low socioeconomic class that is afflicted by both inferior nutrition and inferior housing. It can be hypothesized that, among urban children, those with FeD—and an inherent increase of EP—may have a tendency to absorb more Pb and, thus, to have BPb in the higher range of the "norm." In this case, the association of increased EP and BPb observed within the normal BPb range would be spurious, in reality reflecting only FeD. However, several arguments make this hypothesis implausible. First, EP is clearly correlated with BPb, with an exponential increase that continues at the same rate even when these reach extremely increased values. This relationship has been clearly documented not only in children (9–11), among whom FeD may occur frequently, but also in adult males (12, 13), among whom FeD is quite rare. Second, in the children studied by Roels and co-workers (26), the mean age was 10 yr (an age when FeD is rare); age and socioeconomic class of both groups were the same, and the only difference was in the proximity to a smelter, with the resulting increase in BPb. Third, because FeD is by far the most common cause of non-Pb-related increase of EP in children, its frequency is essentially identical to the value of *C* computed by probit analysis. At 2 SD, this was 2.4% in the present study (a value comparable to that observed in surveys of urban children of comparable age)—a frequency too low to influence the estimate of EP threshold in any significant manner. Fourth, in the present study, samples from 593 children age <2 yr were excluded to minimize the incidence of FeD, which is at its highest value in this age group. When the relationship between BPb and EP was analyzed, either in this group separately or by adding these samples to the rest of the data, the estimates of threshold remained essentially unchanged. Yet, the incidence of FeD in this group of children was substantially higher because the probit technique at 2 SDs yielded a *C* value of 4.5%. It appears, therefore, that the increase of EP is a direct effect of the increase in BPb. EP increases exponentially with a linear increase in BPb. A linear increase in BPb, in turn, reflects an exponential increase in body burden of Pb (7). Thus, the exponential increase in EP reflects an exponential increase in the dose of Pb.

Our study demonstrates a threshold BPb above which the frequency of an increase of EP is greater, which is well within the range of BPb currently called normal. Our observations, together with the reports of inhibition of AmLev dehydratase (22), confirm the hypothesis of Patterson that, even at the low level of exposure of the general population, adverse biological effects of Pb can be demonstrated if searched for with adequate techniques.

The clinical relevance of AmLev dehydratase inhibition is not limited to its effect on heme synthesis. The secondary accumulation of its substrate, AmLev, can affect the neurological system in a manner similar to the action of porphobilinogen (31, 32). The clinical symptomatology of Pb poisoning is close to that of acute intermittent porphyria, a disorder with accumulation of both AmLev and porphobilinogen (33). The accumulation of AmLev is reflected by its increased excretion in the urine, which is exponentially correlated with BPb (24). Selander and Cramer reported that urinary AmLev excretion accelerated at BPb >40, a value at the time accepted as the upper limit of normalcy (24). Because it is well established that AmLev dehydratase is significantly inhibited at BPb <40 (22), the failure to acknowledge any accumulation of AmLev at low doses of Pb made it necessary to postulate a "reserve capacity" of the en-

zyme (7). Yet it is apparent that the exponential increase in urinary AmLev starts in reality at BPb well below 40, once this *a priori* restriction is removed.

The effect of Pb on EP reflects its interference with the last step of the heme biosynthetic pathway, at the level of the mitochondria in the erythrocyte precursors in the bone marrow. Thus, the elevation of EP has the same biological meaning as increased urinary AmLev, that is, damage to a biochemical step beyond any hypothetical reserve capacity. The evidence of damage to a mitochondrial function is obvious in the erythrocyte because blood is the easiest tissue to sample. However, the affinity of Pb is not limited to the mitochondria in the bone marrow but also to those in other tissues (4). Inhibition of heme synthesis affects all body tissues in which heme is the prosthetic group of the cytochrome system. Accumulation of metalloporphyrins further inhibits mitochondrial function (34). The effect of Pb on protoporphyrin has been shown to occur in neural tissue cultures, where its increase is most obvious in the glial cells (35). Damage to glial cells is a common autopsy finding in childhood neuroencephalopathy (36).

The biochemical damage underlying certain clinical symptoms of Pb toxicity has been clarified recently [such as the inhibition of guanine aminohydrolase in saturnine gout (37) or the inhibition of adenylcyclase in neuroconductive disturbance (38)]. Biochemical damage to the heme synthetic pathway, which is reflected by inhibition of AmLev dehydratase and elevation of EP and has the potential of mediating neurological toxicity, cannot be discounted solely because it is already apparent at "normal" BPb, and it is not easily associated with clinically obvious symptoms. It is now clear that the BPb levels currently accepted as normal reflect instead a high level of environmental pollution (1–3). In fact, subtle signs of neuropsychological disturbance have been recently demonstrated at levels of exposure to Pb in the usual range in apparently normal suburban children by use of well-controlled experimental design (6).¶

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¶ Yet the recent National Health and Nutrition Examination Survey (NHANES II) still demonstrates that, among American children age <5 yr, the frequency of individuals with BPbs at >30 is 4%. Among urban children, the frequency is 7.2%; in those residing in the central city, it is 11.6%. The average BPb (and the frequency of children with BPbs of >30) is inversely correlated to family income (Annest, J. L., O'Connell, D., Roberts, J. & Murphy, R. S., American Public Health Association, Los Angeles, CA, November 1–5, 1981, Abstr. 2125).

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