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CLINICAL AND LABORATORY EVALUATION OF LEAD INTOXICATED CHILDREN

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CLINICAL AND LABORATORY EVALUATION OF LEAD INTOXICATED CHILDREN

by

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A thesis submitted to the faculty
in partial fulfillment of the
requirements for the degree of

Doctor of Medicine

Yale University School of Medicine
Department of Pediatrics
New Haven, Connecticut

1970

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Introduction

The problem of lead ingestion, a major one to physicians and community leaders, has achieved increased prominence in the last several years. It is a problem that can be approached from many directions: clinical, biochemical, psychological, sociological, and political. The purpose of this paper is to look at some of the many clinical and biochemical parameters available for evaluating a child suspected of lead ingestion and see which one, if any, would be valid for mass screening and which, as currently used, add little to the evaluation of the patient.

Background material, including the magnitude of the "lead problem", pathophysiology of lead ingestion, and pertinent studies from the literature, will be discussed first, followed by our materials and methods, results and conclusions. Fortunately for the children there were insufficient numbers of lead intoxicated patients to draw many definite conclusions, however much of the data is quite suggestive and should add to our ability to evaluate lead ingesters.

Historical Perspective

General:

The exact magnitude of lead ingestion is not known. In 1955-1963 in New york City 3% of the 60,000 reported pediatric poisonings were due to lead (1) and this has probably increased significantly with the recent vigorous campaign. It is predominantly a disease of urban slums with an estimated 15-25% of preschool children having increased absorption with about one-third of these having signs or symptoms of intoxication (2,3,7). Pica, which starts in the second year and extends through the fourth year of life is usually a key symptom in bringing the child to a doctor's attention (5). It is estimated that 10-30% (1,4,17) of children with pica have symptoms or signs of lead intoxication. Conversely,history of pica could only be elicited in 60% of cases when initially screened by an M.D. (6)

Signs and symptoms:

The signs and symptoms, which usually occur only after 3-6 months of ingestion, are protean (8). The symptomatic patient may present with insidious onset of abdominal pain with constipation or vomiting; anorexia, irritability, or symptoms of anemia. These may progress to clumsiness, lethargy, and even convulsions and coma (6, 8,9,). Physical exam is usually unremarkable except for pallor

and, in cases of severe intoxication, signs of increased intracranial pressure. The encephalopathy is the most feared complication as at least 25% of survivors of encephalopathy sustain permanent brain damage such as seizures, mental retardation, specific learning deficits, or behavioral disorders(10).

Fate of lead:

Normally, the intake and output of lead are equal-what is ingested and inhaled is excreted in the urine and feces (12). Usually less than 1 mg/day of lead is ingested 90% of which is passed out in the stool (2). Kehoe, in his balance studies, states that the safe levels of ingestion are less than 1.3 mg/day. At an ingestion rate of 1.3 mg/day toxicity is reached in 7.5 years, at 2.2 mg/day toxicity is reached in 3.9 years and at 3.2 mg/day only 0.64 years are required to reach toxic levels (13). Therefore, those who consistently take in more than 2-3 mg/day require about 6 months before lead levels become toxic. Of interest is the fact that the lead problem is worse in the summer, a fact attributable to an increase in Vitamin D in children exposed to the sun which leads to an increased GI absorption of lead (3).

Once in the body the lead goes first into the soft tissues and quickly leaves and is transferred into bone. The tissues most affected are liver, kidney and then brain with levels reaching 30 times those of controls (14).

Normal blood levels may again appear by 6-12 months after ingestion stops, when most of the lead is in the bones (2). However, a great deal of lead may be present and things that mobilize calcium may mobilize lead. Some of these include: low phosphate, intercurrent infection, increased parathyroid hormone, increased Vitamin D, acid-base imbalance, or bone disease (2,15,16). The elimination of these accumulations of lead is primarily through the urine. Kehoe (12) showed that the time required for elimination was at least two times that required for accumulation and was proportionately longer as the period of accumulation lengthened. One would then see urine levels decline rapidly after ingestion and then fall more gradually as the accumulated load is excreted.

Lead:

Lead levels in blood and urine have been widely used to evaluate potentially intoxicated patients. Since only blood levels were used in our study the discussion will be limited to them.

Of primary importance in measuring blood levels is is that whole blood and not serum be used. In most studies 90-95% of lead is said to be bound to the red blood cell (11,14,15,18,19). It is bound as a sol and there is no inhibition of lead uptake by metals such as calcium (19). Implicit in the fact of red cell binding

is that a patient's hematocrit must be taken into account in blood lead levels (usually given in mg/100 gm of whole blood).

What is considered a normal blood lead is quite variable. In most of the studies discussed below it is not clear whether or not blood leads were corrected for hematocrit and if so to what hematocrit they were corrected to, hence this adds to the confusion of "normal values". Although the methods of lead determination are not mentioned most of the methods were probably the Dithizone-colorimetric method which can detect to 0.2 ug + or - 0.1 ug. (20)

Various upper limits of normal in mg Pb/100 gm whole blood are:0.02 (14), 0.036 (17), 0.04 (10,15), 0.05 (22), 0.06 (1,5,8,11,21). 0.06 seems to be the one most widely used. Values for definite toxic levels with risk of encephalitis was 0.08 (5,6,10) - 0.10 (2,4) and above. However, Bradley (22) in his study of 462 patients had 12 of 27 patients with central nervous system symptoms, 15 of 80 with gastrointestinal symptoms and 22 of 49 with stippled red cells with values below his upper limit of normal of 0.05 mg%. Chisolm (15) points out that lead levels are affected by many factors including: hematocrit, intercurrent infection, coincident bone disease, current or recent administration of chelating agents, current or

excessive absorption of food, and time interval since excessive absorption ceased. For example, in a child with a blood level of 0.04- 0.08 mg% it is difficult on the basis of blood lead value alone to distinguish between temporary dietary change and increased body burden. He thus recommends that other parameters of lead's metabolic toxicity (e.g. urinary d-ALA) may be used as a more accurate index of soft tissue lead. Berman (14) further comments that blood lead levels indicate the degree of lead absorption but that these are of value only in acute cases since blood is rapidly cleared of lead. He found that blood levels in chronic cases are not remarkable. It seems, then, that blood levels, once the standard for lead toxicity, may be not entirely dependable in that: a) values are not always standardized for hematocrit, b) symptomatic and even toxic patients may have normal values and c) chronic ingesters may have normal values.

Toxic effects of lead

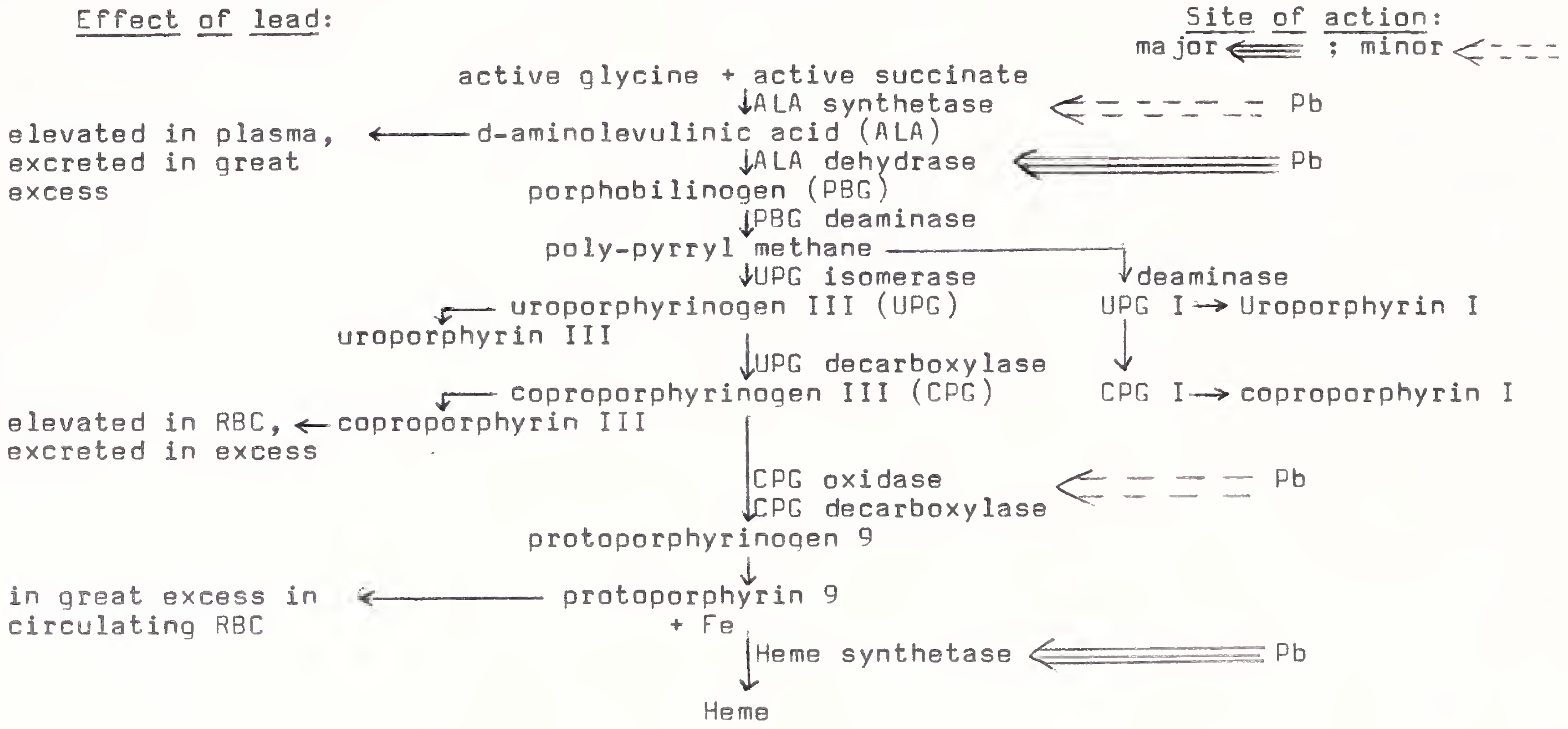
Heme synthesis and anemia:

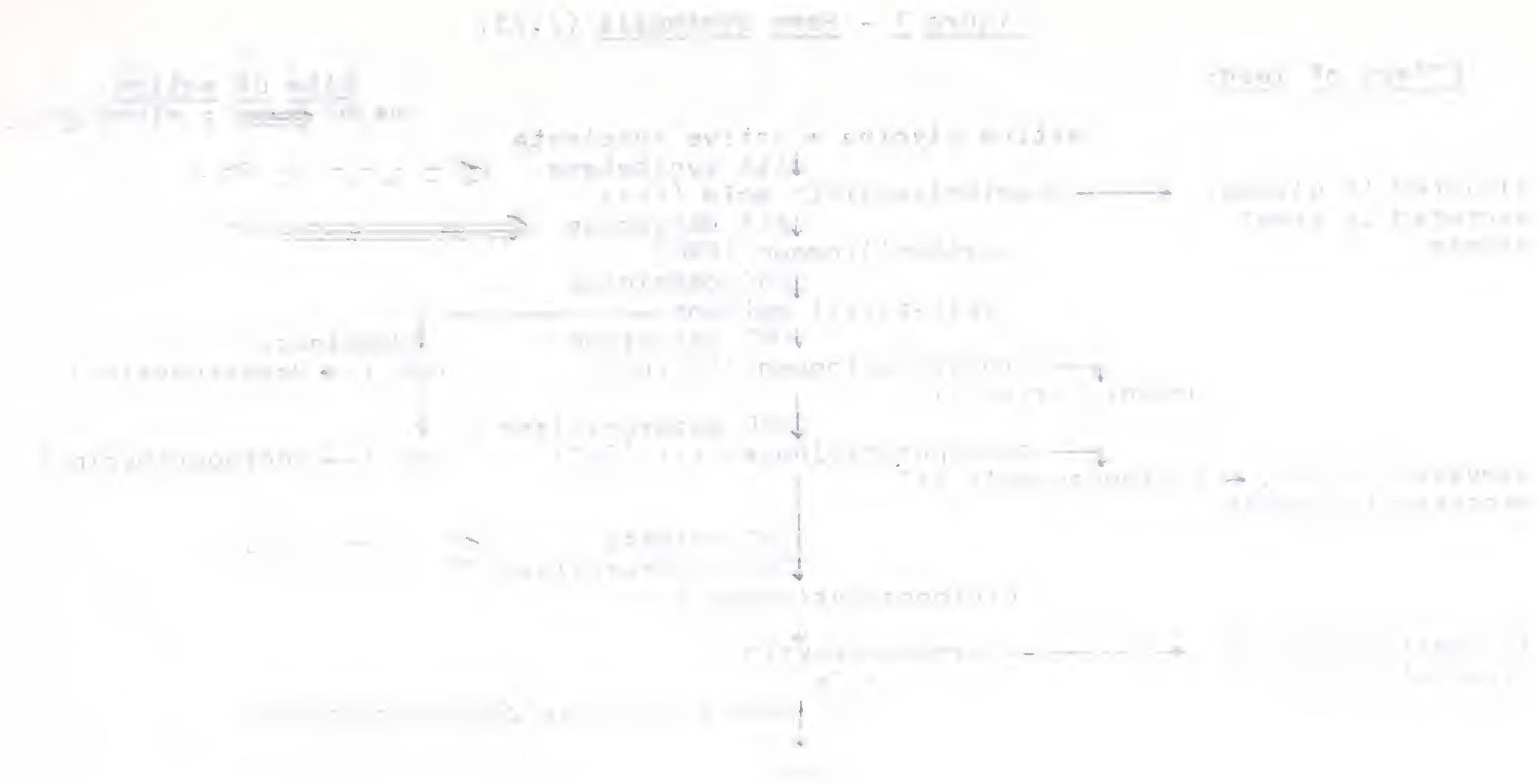
One of the common findings in lead ingesters is anemia. Since iron deficiency is also fairly common in this age group (4,6) it will be worth while to discuss some of the mechanisms of lead anemia and the relationship to iron metabolism.

Figure 1 is a summary of heme synthesis. The currently accepted sites of action of lead are indicated (2,23, 24). Lichtman and Feldman (23) showed that ALA-dehydrase activity is greatly reduced thereby producing and excess of delta-amino-levulinic acid (ALA). The lead probably inactivates the sulfhydryl groups as this inhibition is reversed by preactivating the system with reduced glutathione. The second major step inhibited is the incorporation of iron into protoporphyrin 9 which accounts for the excess of protoporphyrin. Minor effects of lead on CPG oxidase or CPG decarboxylase is also seen, leading to an excess of coprporphyrin III. Lead probably also decreases ALA synthesis but to a minor degree. Chisolm (25) has quantified the abnormal heme precursors and the reader is referred to this for specifics.

Serum iron and percent saturation may be high or low. Iron absorption, transport and storage are unaffected in lead intoxication (31). Lichtman and Feldman studied effects of iron deficiency on heme synthesis and found no

Figure 1 - Heme Synthesis (2,23)





effect of iron deficiency on ALA dehydrase. They found an *in vitro* decrease of porphyrin synthesis in iron deficiency that may have been due to a decrease in PBG deaminase or isomerase activity. Hence, iron levels may be high in lead poisoning from inhibition of iron incorporation into heme but levels are usually low due to iron deficiency (22). The iron deficiency itself, however, has little effect on heme synthesis.

Other aspects of red cell dynamics are also affected. Severe anemia with severe erythrocytic hypoplasia of the bone marrow, reversible after treatment is reported in one boy (26). Leiken and Eng (29) did Fe^{59} and Cr^{51} red cell kinetic studies on seven patients $1\frac{1}{2}$-10 years old who had iron resistant anemia, pica, increased lead levels and X-ray evidence of excess lead ingestion. All patients had a normal or increased serum iron and significant stainable iron in the marrow. In patients with less than a one year history of ingestion a hemolytic picture was seen: increased iron incorporation, increased erythrocyte iron turnover, decreased RBC survival, and increased erythroid elements (decreased myeloid/erythroid ratio). In patients with a more than one year history a hypofunctioning marrow picture was seen: decreased iron incorporation, decreased erythrocytic iron turnover, normal RBC survival, and a decreased erythroid

element of the marrow (increased M/E ratio). Other reported findings in lead poisoning are: decreased osmotic fragility, increased mechanical fragility (27, 31), occassional positive Coombs test (31), normal haptoglobin levels (30,31), low hemoglobin,red count, MCV,MCH, MCHC, low percent saturation of iron binding capacity (28), and increased reticulocytes (4,28). It is noted that these findings are similar in iron deficiency (i.e. low hemoglobin, red count, MCV, MCH, MCHC, percent saturation of iron binding capacity) and it is to be remembered that iron deficiency often occurs in these patients. Differential may be made by the increased reticulocytes in plumbism compared to untreated iron deficiency, and mainly the finding of abnormal heme precursors in plumbism.

ALA:

Delta-aminolevulinic acid, one of the heme precursors elevated in lead intoxication, is fairly readily measurable in plasma or urine and is often one of the criteria used in evaluating lead intoxicated patients. The most widely used method, the Haeger-Aronson method, is sensitive to plasma ALA within 5% of ALA values above 0.03 mg/100 cc plasma or serum. Normal values, which depend on individual equipment and standard curves, are: 0.019 +or- 0.004 mg% (11) and 0.0114 +or- 0.0053 mg% (32). Chisolm, in a different method, had values of 0.0056 +or- 0.00019 mg% (33).

In one study Chisolm (55) showed higher plasma ALA levels in patients with lead encephalopathy than in patients with acute lead intoxication but without encephalopathy. Both of these groups had higher levels than in totally asymptomatic patients with increased lead absorption. Feldman et al (32) have studied three groups of patients, broken down by clinical classification, and has studied the ALA and lead values of these groups. Group I had elevated Pb (above 0.06 mg%) but no other clinical or laboratory signs of toxicity. Group II had elevated Pb and other laboratory evidence, and Group III had elevated Pb, other laboratory evidence, and encephalopathy. The results were:

Group	Pb-mg/100ml blood	ALA- mg%
normal	less than 0.06	.0114 +/- .0053
I	.069 +/- .017	.0106 +/- .0062
II	.083 +/- .025	.0371 +/- .0014
III	.196 +/- .102	.0959 +/- .0491
	p less than .05	p less than .005

There is good significance between the different groups with a greater degree of overlap in lead levels compared to ALA levels. The graph of serum ALA vs. Blood lead "suggested a positive relationship between the variables with wide scatter." The authors concluded that elevated blood lead levels indicate exposure to and increased absorption of lead and they do not necessarily indicate lead intoxication. On the basis of the fact

that the ALA values ofthe groups showed good separation and that all patients with stigmata of lead intoxication had elevated ALA levels he concluded that "the serum d-ALA level proved to be an extremely sensitive indicator of toxicity and appears to be useful in selecting patients who may require hospitalization and detoxification with chelating agents (32)."

Basophilic stippling:

Basophilic stippled red cells are often seen in peripheral smears of lead intoxicated patients. From 30% (5) to 60% (6,32,36) of patients are reported to have them although 800 stippled RBC/million RBC is said to be a normal finding (35). The granules are actually aggregations of RNA surrounding mitochondria (11, 25,27). These cells differ from mature cells in the following ways: 1) mature cells do not contain mitochondria and hence connot synthesize heme precursors, while these cells have mitochondria and can synthesize the precursors; 2) mature cells consume little oxygen while basophilic stippled cells have higher consumption than mature cells and even higher than reticulocytes (25); 3) these cells contain more cytochrome C and adenine nucleotide than a mature cell (11). Hnece these cells are very active metabolically. The basophilic stippled cells are probably destroyed in an increased rate by the reticuloendothelial

system as splenectomy or reticuloendothelial blockade with trypan blue produces a marked increase in the number of stippled cells in guinea pigs (28). These cells are different than reticulocytes and the number of reticulocytes does not correlate with the number of basophilic stippled red blood cells (4).

The usefullness of these as a criterion for lead intoxication is questionable. Firstly, they are, as shown above, at best found in 40-60% of lead intoxicated patients. They do not correlate with intensity of exposure and may be seen in other disorders such as anemia, leukemia, thalassemia, or exposure to other compounds such as benzene, aniline, or carbon monoxide (27, 31). There is also no value in quantifying the number of stippled red cells as this can be changed by different staining and drying procedures. It is therefore suggested that the finding of these cells should arouse suspicion of lead intoxication and that more reliable studies should be subsequently done (34).

Flourescent red cells:

As indicated in Figure 1 there is an excess of erythrocyte protoporphyrin in lead poisoned patients. A property of this porphyrin, red flourescence under ultraviolet stimulation at approximately 400 mu, is the

basis for another test of lead poisoning- flourescence of erythrocytes, The flourescent cells are called flourescytes.

The basic technique,described by Whitaker and Vietti (38) and subsequently modified, involves placing a drop of heparinized blood on a slide, suspending it in saline, looking at it under UV stimulation of approximately 400 mu, and counting the percent of flourescytes. The counting must be done rapidly as the flourescence fades within 15 seconds when a mercury vapor source is used, which is the common light source. Cripps et al (40) reported longer flourescence with an iodine tungsten source but the papers reviewed here used the mercury vapor lamp. Whitaker and Vietti (38) found no flourescytes in normal children, 0-50% in iron deficient children, 25-100% in 12 treated lead poisoned patients, and 75-100% in 7 untreated patients. Nelson's group (39) studied 869 patients with anemia but with no lead poisoning and 33 patients with proven lead poisoning. Of the 869 with anemia 98.4% had less than 1% flourescence and only 2 patients had greater than 5% flourescytes. Of the 33 patients with proven lead poisoning all but one had flourescytes (but this patient had them on repeated testing three days later) with 31 patients having more than 10%. He, therefore, called less than 1% normal,

and greater than 10% lead poisoning with 2-10% being the gray zone. Using these limits of normal he found excellent correlation with % flourescytes and lead excretion after EDTA (p less than .005) and an excellent correlation with erythrocyte protoporphyrin, the likely cause of the flourescence. He also showed that in 10 patients followed up after treatment all had disappearance of flourescence with a mean time of 9.7 months. The lower ranges of flourescence in Nelson's paper is due to a second red excluding filter not used by Whitaker and Vietti. Both studies seemed to imply that this is a good screening test. Nelson et al gave the normal range as listed above and recommended work up of anyone with greater than 5% flourescytes. It must be remembered that in initial screening 1 of 33 patients with proven lead poisoning had less than 1% flourescence-a false negative in 3% of cases. Other editorials on lead poisoning (10) comment that this is probably a good technique for those skilled in it but that it is not potentially useful for general hospital laboratories,

Lead hemoglobin:

A final hematological finding in approximately 50% (31) of intoxicated patients is that of lead hemoglobin. Charache (37), doing hemoglobin electrophoresis at pH 8.6, found that in 10 of 24 lead poisoned patients

there was a component that migrated in front of the major hemoglobin component. This was called lead hemoglobin (Hb-Pb) which seems to be identical to Hb-A_3 which normally is seen in senescent red cells. The mechanism of its production is not known but it is not seen in iron deficient patients without lead poisoning. If the Hb-Pb represents Hb-A_3, which it most probably does, it may indicate premature senescence of the red cell in lead poisoning.

Effects on renal function:

The reports of the effects of lead on the kidney have varied. Earlier reports of a twenty year follow up of lead poisoned children in Queensland, Australia stated that 101 of 165 patients who died had "nephritis or renal sclerosis" (41). Teper, however, in his twenty year follow up of 42 lead intoxicated patients in Massachusetts found no evidence of chronic renal disease (41). Illis (42) found abnormal blood ureas and creatinines in lead workers who had increased lead absorption (elevated lead in urine). Henderson (43) found higher bone lead in patients with chronic renal disease of unknown origin than in patients with either no renal disease or with renal disease of certain origin other than lead poisoning. Increased incidence of gout in chronic lead ingesters, by a mechanism to be described below,

has also been reported by various authors (44, 45, 46). It therefore seems that lead ingestion may lead to an increased incidence of chronic renal disease.

A second important question is whether or not there is evidence of change of renal function in acute lead intoxication. Smith (36) reported that albuminuria or glycosuria is present to a slight degree in 30% of childhood lead intoxications. Chisolm (47) reported aminoaciduria, glycosuria, and hypophosphatenia, the Fanconi syndrome- in 9 of 23 lead intoxicated patients before EDTA treatment. In the remaining 14 patients there was varying degrees of aminoaciduria corresponding to the clinical severity of the intoxication. Hence it seems from these reports that a change of renal function is readily apparent in 1/3 to 1/2 of the lead poisoned patients. It must be remembered that EDTA may cause aminoaciduria itself (16) but in these studies the urine tests were done before administration of EDTA. However, in Chisolm's study (47) the aminoaciduria cleared after 24 hours of administration of EDTA.

There have been varied descriptions of the site of action of the lead in nephropathy. Intranuclear inclusions were seen in the proximal tubule cells (2,11,45,47,48), as well as in the nuclei of other body cells- liver, pancreas, and brain (2). Electron micrographs (49) have

shown changes in the epithelial cells identical to those of lipoid nephrosis in four humans with proteinuria and lead poisoning. The tubular lesions are said to be reversible (11,47) however in chronic poisoning vascular lesions with development of nephrosclerosis may occur (11).

The pathophysiologic changes involve primarily the tubules. Chisolm (47) that while normally 95-99% of filtered amino acids are reabsorbed less than 90% are reabsorbed in acute plumbism. The incidence of gout is remarkably elevated in patients with lead nephropathy. Emmerson (46) showed that 16 of 33 patients with chronic lead nephropathy had gout while only 1 of 18 with non-lead nephropathy had it. Of interest is that 7 of 19 females with lead nephropathy had gout. Uric acid balance studies by Emmerson (44) and Ball (45) showed: 1) no increase in uric acid production; 2) normal tubular reabsorption of uric acid; 3) marked defect in tubular secretion of uric acid; 4) fall in urate clearance out of proportion to the decrease in clearance of creatinine and inulin. Number 3 above appears to be the mechanism of the increased incidence of gout in chronic lead nephritis.

In acute lead poisoning, then, one may see aminoaciduria, glycosuria, and proteinuria from tubular lesions and possibly glomerular lesions which may be reversible.

In chronic lead nephropathy, from prolonged ingestion and/or irreversible damage to the tubules, a decreased tubular secretion of uric acid is seen with elevated serum uric acid levels. Elevated blood urea and creatinine levels, also evidence of chronic renal disease, may be seen.

Encephalopathy:

Encephalopathy is one of the feared complications of lead ingestion and intoxication. Acute encephalopathy has a mortality rate of 20% or more (2) while at least 25% of the survivors sustain permanent brain damage such as seizures, mental retardation, specific learning deficits, or behavioral disorders (10). The lesions caused by the lead are due to increased capillary permeability and petechial hemorrhages which cause death of neurons and formation of cerebral edema (3). The patient will have symptoms of encephalopathy, along with increased cerebrospinal fluid pressure, protein and cells, usually lymphocytes (4).

X-rays of bones and abdomen:

Radiologists often look for evidence of lead ingestion in the abdomen and ends of long bones. Radiopaque flecks in the abdomen are seen in approximately 50% of cases (10,36). Since lead is radiopaque these are thought to represent lead, but non-lead materials may

show (8). The nature of the lead line is not exactly clear. Kissane and Smith (63) suggest that it is due to subepiphyseal zone of provisionly calcified osteoid in which metaplasia to form cancellous bone is retarded. Chisolm (2) says that storage of excess lead in bone produces the characteristic radiologic changes in the metaphyses of long bones and that the broad band reflects the densely packed, irregular trabecular structure of bone being formed in the presence of excess lead. Anderson (65) comments that dense lines are often seen in abnormal absorption and storage of heavy metals. Others (8,16,64) say it is due to deposition of lead in the epiphyses and "must be distinguished from growth arrest lines (8)". Sartain's (51) study of X-ray findings in acute and chronic lead ingesters and in normals showed the following: chronic ingesters- 2 definite positives, 7 equivocal, 5 normals (36%0; acute ingesters- 1 positive, 3 equivocal, 10 normal (72%); normal controls- 3 equivocal, 11 normal (79%). He showed that it usually requires six months of ingestion for lead lines to appear, that lines may be absent early in the course, and that false positives may occur. The logical conclusion was that x-ray findings should be a secondary consideration and that their absence should not preclude further study nor should their presence be taken as proof of intoxication.

Miscellaneous:

Various other effects of lead ingestion have been looked at. Kopito (52) studied the lead concentration of hair. Since absolute amounts of hair lead varied with environment two or more adjacent hair segments, 5-10 mm long, were studied. An increased concentration was seen near the scalp in acute ingestion, while a generalized increase was found in chronic ingestion. He found that hair lead correlated well with urine and blood lead. Sanstead (53) studied thyroid function in 24 patients with excess lead ingestion from moonshine whisky and found injury to iodine trapping and concentrating mechanisms. Muro (54) studied chromosomal damage in rats fed lead salts. Those fed salts for prolonged periods developed renal adenomas, renal adenocarcinomas, decreased fertility, increased frequency of abortions and miscarriages, and increased congenital malformations. Examination of leukocytes from 10 male and 10 female rats fed 1% lead acetate for two weeks showed an increased number of abnormal chromosomes compared to controls. These included gaps, breaks, and fragments. It was seen earlier in the renal section that lead morphologically affects nuclei of human cells, therefore, chromosome studies like these may become increasingly significant in the future.

Of passing interest is the relationship of the symptoms of lead poisoning to those of acute intermittent porphyria (AIP) (58) both of which have abnormal por-

phyrin metabolism. Both have GI symptoms of abdominal pain, constipation, and vomiting. The nervous system signs differ: plumbism has central signs of motor incoordination, and lethargy while in AIP psychiatric symptoms are more common. Both have increased urine ALA, however AIP has a marked increase in urine porphobilinogen compared to lead poisoning while urine coproporphyrin is markedly increased in plumbism compared to AIP. It is therefore interesting to speculate on the causal effect of the abnormal porphyrin metabolism on symptoms of lead intoxication.

This study did not deal with the treatment of lead intoxication and the reader is referred to Chisolm's (55) article on this subject.

The relative merits of the different parameters, the determination of which is the purpose of this paper, was difficult to ascertain from the literature as few authors looked at many parameters at once. One paper (56) chose blood lead over urine lead, urine ALA and basophilic stippling, with hemoglobin determination being of no merit. Another group (57) chose serum ALA over urine ALA, urine lead or other urine porphyrins. The merits of the other tests, mentioned above, will be reevaluated in the discussion of our results later in the paper.

Materials and Methods

Patients:

There were 66 patients studied- 35 males, 28 females with three patients being done on 2 separate occassions. The mean age was 32.5 months with the distribution as noted in Figure 2. The patients were selected on the basis of their being potentially lead intoxicated. These included patients with: previous history of lead ingestion; those who were related to lead ingesters; patients with a questionable history or definite history of pica; and patients with unexplained central nervous system symptoms such as lethargy.

Samples:

From each patient, whenever possible, 25-30 cc of blood were drawn. 12 cc were sent to the state laboratory for lead analysis, 8-10 cc were sent to the clinical chemistry lab for iron, iron binding capacity, and uric acid, and 5-10cc were sent to our lab for tha other analyses. Since blood samples were often difficult to obtain from the young patients; at times insufficient amounts of blood were sent to our laboratory hence we frequently had insufficient samples on which to assay for ALA.

Urine samples were difficult to obtain because of the age group and the fact that many patients were outpatients and were unable to void while at clinic. When

Figure 2- Patient population

Age in months	Number of patients
0-10	1
11-20	12
21-30	21
31-40	14
41-50	8
51-60	3
61-70	2
71-80	2
81 and above	1

The mean age is 32.4 months. Three patients were done on two separate occasions.

Sexes: Males- 35; Females- 28.

possible 5-10 cc were obtained and these were tested as described below.

Lumbar punctures were done on patients with elevated blood lead levels. Samples were obtained by the house officers.

All blood tests, with the exception of serum ALA, hemoglobin electrophoresis, and flourescytes, were done within 6-8 hours of collection during which time the whole blood was refrigerated. The plasma and cells were separated within 6-8 hours of obtaining the samples and the cells were refrigerated for flourescyte screening, done within 24 hours, and the serum was frozen for ALA determination, done within 2 hours of thawing the sample. The aliquot of cells for electrophoresis was washed and refrigerated until the electrophoresis was done, which was usually done on a once per week schedule.

History:

All the patient's charts were reviewed and pertinent history was obtained from them including history of pica, other hematological or pertinent medical history, and X-ray reports.

Laboratory studies:

Blood: studies done by the hospital laboratory were:

1) Red and white blood counts were done in duplicate by Coulter Counter.

2) Serum iron- released iron measured as iron-bathophenathrolone complex color reagent. Iron binding capacity (IBC)- protein saturated with iron and measured as iron.

Normal values: Fe(ug %)- males- 60-180 females-50-170

IBC(um %)- males and females- 248-422

3) Uric acid by urease method.

Normal values (mg%)- male-4.5-6.5 females 2.5-5.5

Studies done by our laboratory:

1) Hemoglobin in duplicate as cyanomethemoglobin

2) Hematocrit- in duplicate by capillary tube

3) Indices- calculated by the standard methods

4) Basophilic stippled red blood cells-

Smear stained with Wright's stain for 3 minutes, Wright's buffer for 6 minutes and the number of stippled cells per 1000 total cells were counted. Distorted cells or cells in an area of the slide with excess stain were not counted.

5) Reticulocytes-

Equal amounts of freshly filtered new methylene blue and whole blood were allowed to stand for 15 minutes. One drop was then smeared on a slide and 1000 cells were counted.

6) Hemoglobin electrophoresis-

Starch block at pH 8.6 allowed to run at least 16 hours. Quantification was done by eluting the individual zones with distilled water and the percent of hemoglobin in each band was calculated after measurement of hemoglobin as described above.

7) Plasma ALA-

Modification of the Haeger-Aronsen (11) method done so

that only 2 cc of plasma needed. Below is listed only the volume differences from the standard methods;

1) 0.1 ml freshly prepared iodoacetamide
   + 1 ml serum
2) 0.5 ml 20% trichloroacetic acid added after 2 minutes
3) 0.5 ml supernatant +
   0.5 ml acetate buffer pH 4.6
   0.05 ml 2.5N NaOH
   0.03ml acetylacetone (omitted from control)
4) 0.75 ml of above solution+
   0.75 ml Ehrlich's reagent II

A standard curve was made with ALA-HCl, A grade from Calbiochem. The gm ALA/gm ALA-HCl was taken into account in the standard curve. The optical density was read in a 1 cm light path by a Hitachi-Perkins Elmer (Coleman 111) spectrophotometer. The standard curve is shown in Figure 3. ALA= O.D./0.6

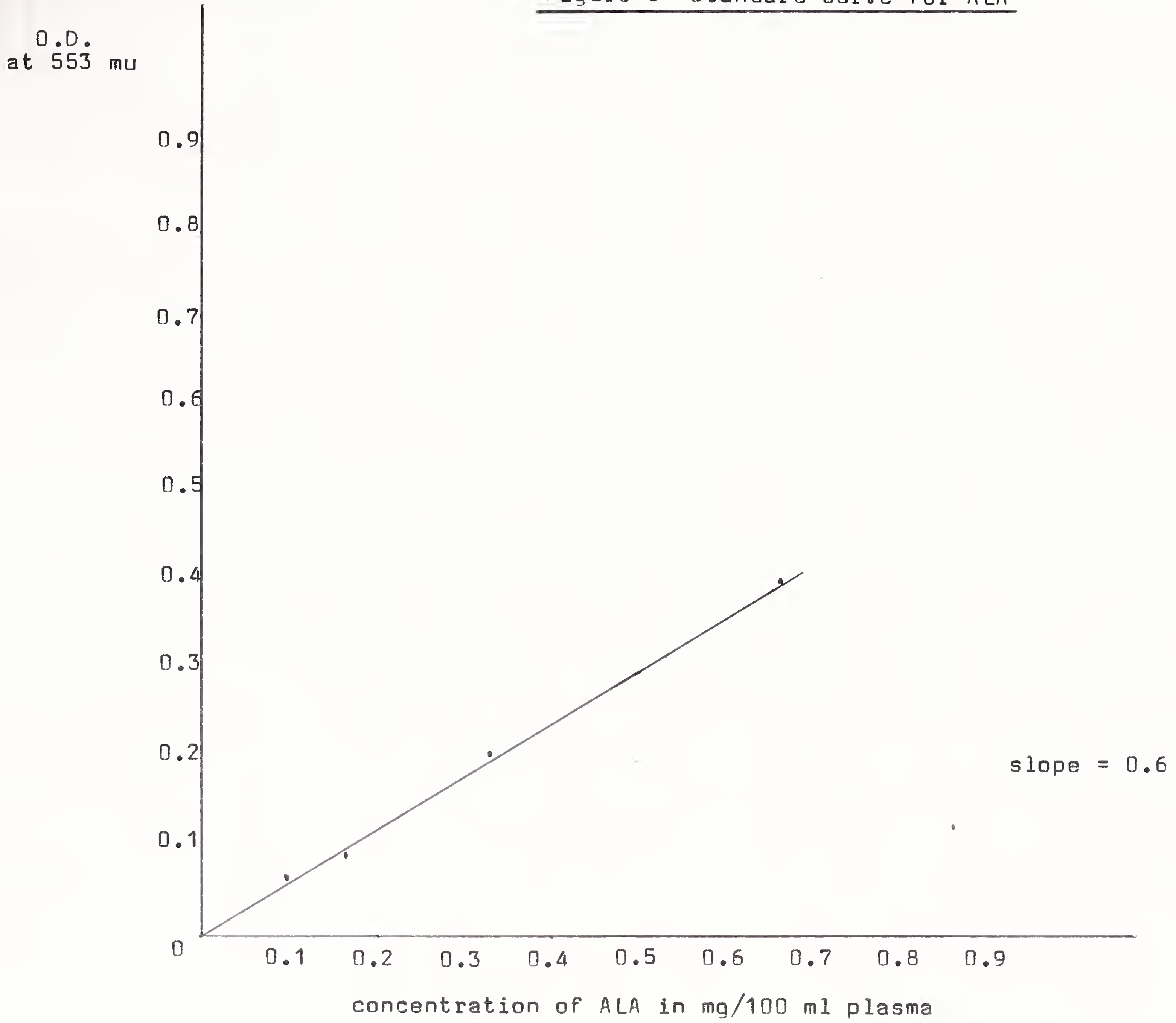
Control; a 250 cc sample of blood bank plasma was divided into 3 cc aliquots which were frozen. One or two of the aliquots were run with each group af ALAs. The control had a mean of 0.037 mg% with a range of 0.035-0.039. Therefore the values differed at worst by only 10% (all values were + or - 5% of the mean).

8) Flourescytes-

A Zeiss microscope with a Vorshaltegrat U. Drossel mercury vapor UV light source was used. A schott BG-12 filter was used with a maximum transmitted wavelength of 400 mu. Magnification was 512x.

Method: One drop of anticoagulated blood was mixed with saline. 50 high power fields were scanned for presence of

Figure 3- Standard curve for ALA



any flourescence. If any was found a second slide was prepared a 5 cells in 40 different fields were counted and the percent flourence was counted. A second red excluding filter was not needed as the cells had distinct flourence with no red tinge on cells fron normal controls.

9) Blood lead-

Assay was done by the State of Connecticut laboratories by a modification of the Cholak method (61). Their upper limit of normal was 0,04 mg/100 ml blood but their values were not corrected for hematocrit.

**In this stidy all of the lead values have been standardized to a hematocrit of 35%.

Urine:

1) Urinalysis-glucose,protein determined with Ames Combistix

2) Urinary amino acids- were done and interpreted by the Yale genetics laboratory.

Cerebrospinal fluid:

1) Protein, glucose, and cells done by hospital laboratory.

Statistics:

Means, standard deviations, correlation coefficients, and probabilities were done by standard statistical methods (60,62). Calculations were done by IBM computer. When two variables were being correlated any unpaired number was omitted from the calculations.

Results

In this section the data will be presented, for simplicity, primarily in graphic and table form. The "raw" data is listed in Appendix 1. The graphs and tables are set up so that the following questions could best be answered: 1) Which of the three, serum ALA, blood lead, or percent flourescytes is a better criterion for evaluating and screening the lead intoxicated patient; 2) Assuming our normal range for ALA is less than 0.095 mg/100 ml plasma, our normal range for lead is less than 0.06 mg/100 ml blood, and our abnormal range for flourescence is any flourescence, how valuable are the other parameters, that is how often do they give true positives, true negatives, false positives, and false negatives.

The normal range for lead is the one suggested by most studies (1,5,8,11,21). All the lead values in our results have been standardized to a hematocrit of 35%. The normal range of ALA differs from the ones cited. However, as will be seen from the data, it arbitrarily appears to be the best cut-off point. In one study (39) the normal value for flourescytes is less than 1%. In our results we arbitrarily used the presence of any flourescence as being significant as quantification is difficult due to the rapid fading of flourescence and we were looking for a rapid screening method.

Figure 4- Correlation of variables

As described in the methods the various parameters were correlated according to standard statistical methods. Only paired samples were used so when two parameters were correlated all unpaired numbers were deleted from that calculation. This accounts for the change of mean and standard deviation when a variable was used in different calculations.

Figure 4

Parameters	Mean	Standard deviation	Number of pairs	Correlation coefficient	Probability
Lead	0.076	0.099			
vs			44	+0.51	less than .001
ALA	0.081	0.025			
Lead	0.066	0.085			
vs			62	-0.51	less than .001
Hemoglobin	10.97	1.98			
Lead	0.067	0.087			
vs			60	-0.21	less than .20
MCHC	31.18	2.64			
Lead	0.061	0.076			
vs			49	-0.29	less than .05
% sat.	14.14	7.64			
Lead	0.061	0.071			
vs			56	+0.14	greater than .20
Retics	1.16	0.61			
ALA	0.081	0.025			
vs			45	-0.35	less than .02
Hemoglobin	10.68	2.15			
ALA	0.081	0.025			
vs			44	+0.02	greater than .20
MCHC	31.19	2.82			
ALA	0.078	0.022			
vs			39	-0.25	less than .20
% sat	14.54	9.64			
ALA	0.078	0.022			
vs			41	+0.45	less than .01
Retics	1.20	0.66			

Figure 5- ALA vs Pb

The graph of ALA in mg/100 ml plasma vs Pb in mg/100 ml blood shows:

1) Assuming lead is the standard of true intoxication with Pb greater than 0.06 being abnormal, then the accuracy of ALA as a test is considered with ALA greater than 0.095 as an abnormal value. Therefore:

with ALA greater than .095 -	Pb greater than .06-	10
	Pb less than .06 -	1
with ALA less than .095 -	Pb greater than .06-	1
	Pb less than .06 -	32

Therefore:

True positives - 10
True negatives - 32
False positives- 1
False negatives- 1

2) Conversely, assuming ALA is the standard of intoxication with ALA greater than 0.095 being abnormal, then the accuracy of Pb as a test is considered with Pb greater than 0.06 as an abnormal value. Therefore:

with Pb greater than .06 -	ALA greater than .095-	10
	ALA less than .095 -	1
with Pb less than .06 -	ALA greater than .095-	1
	ALA less than .095 -	32

Therefore:

True positives - 10
True negatives - 32
False positives- 1
False negatives- 1

Figure 5

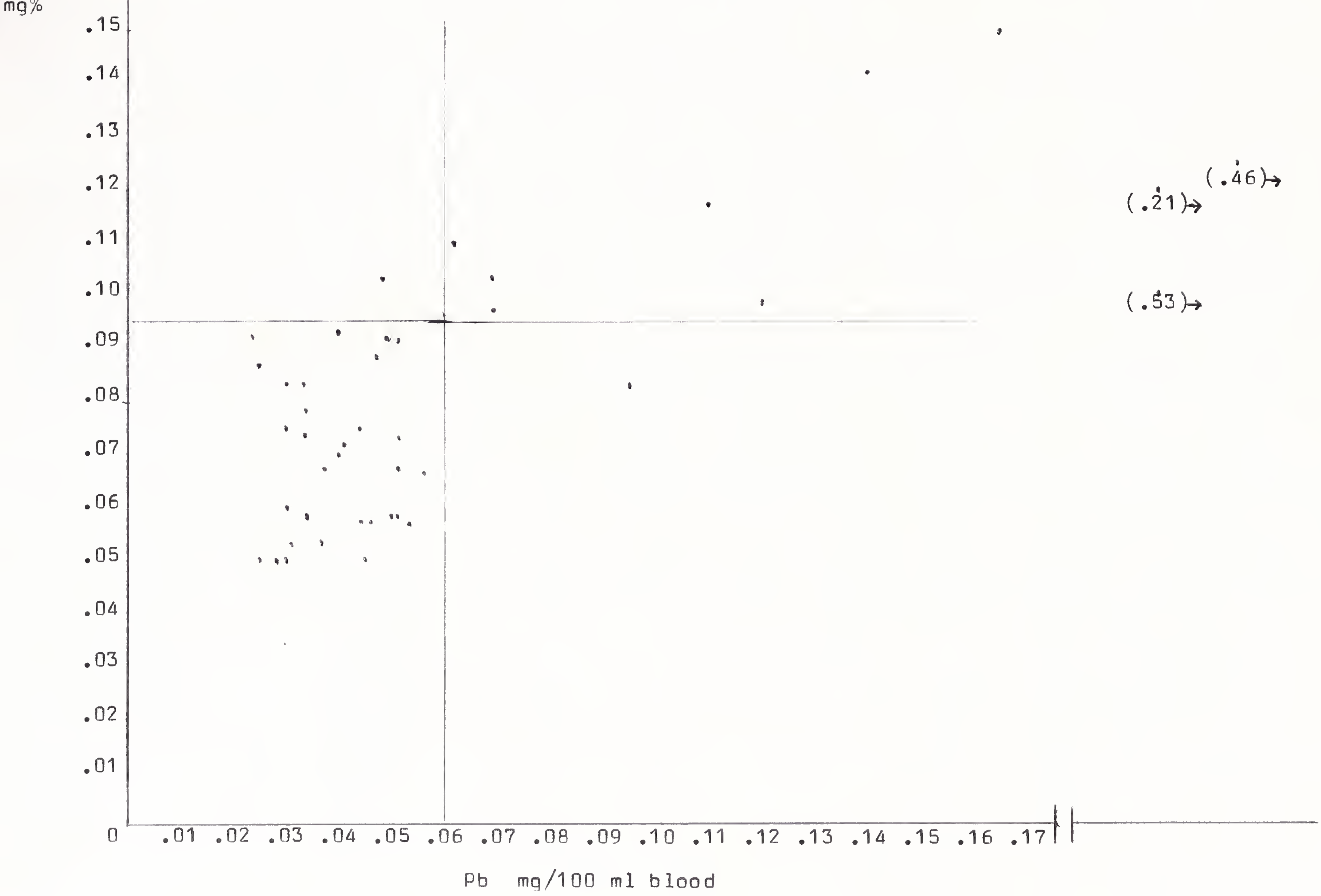

ALA
mg%
.15
.14
.13
.12
.11
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
0
.01 .02 .03 .04 .05 .06 .07 .08 .09 .10 .11 .12 .13 .14 .15 .16 .17
Pb mg/100 ml blood
(.46)→
(.21)→
(.53)→

Figure 6- Flourescytes vs Pb

The graph of flourescytes in percent vs pb in mg/100 ml shows:

1) Assuming Pb is the standard of intoxication with values greater than 0.06 being abnormal, then the accuracy of flourescytes as a test is considered with any flourescence being abnormal. Therefore:

With Pb greater than .06 -	flourescence present- 10 flourescence absent - 2
with Pb less than .06 -	flourescence present- 2 flourescence absent - 46

Or:

True positives - 10
True negatives - 46
False positives- 2
False negatives- 2

2) Conversely, using flourescytes as the standard, and testing the accuracy of lead levels, with the ranges of normal as above, there are:

True positives - 10
True negatives - 46
False positives- 2
False negatives- 2

Figure 6

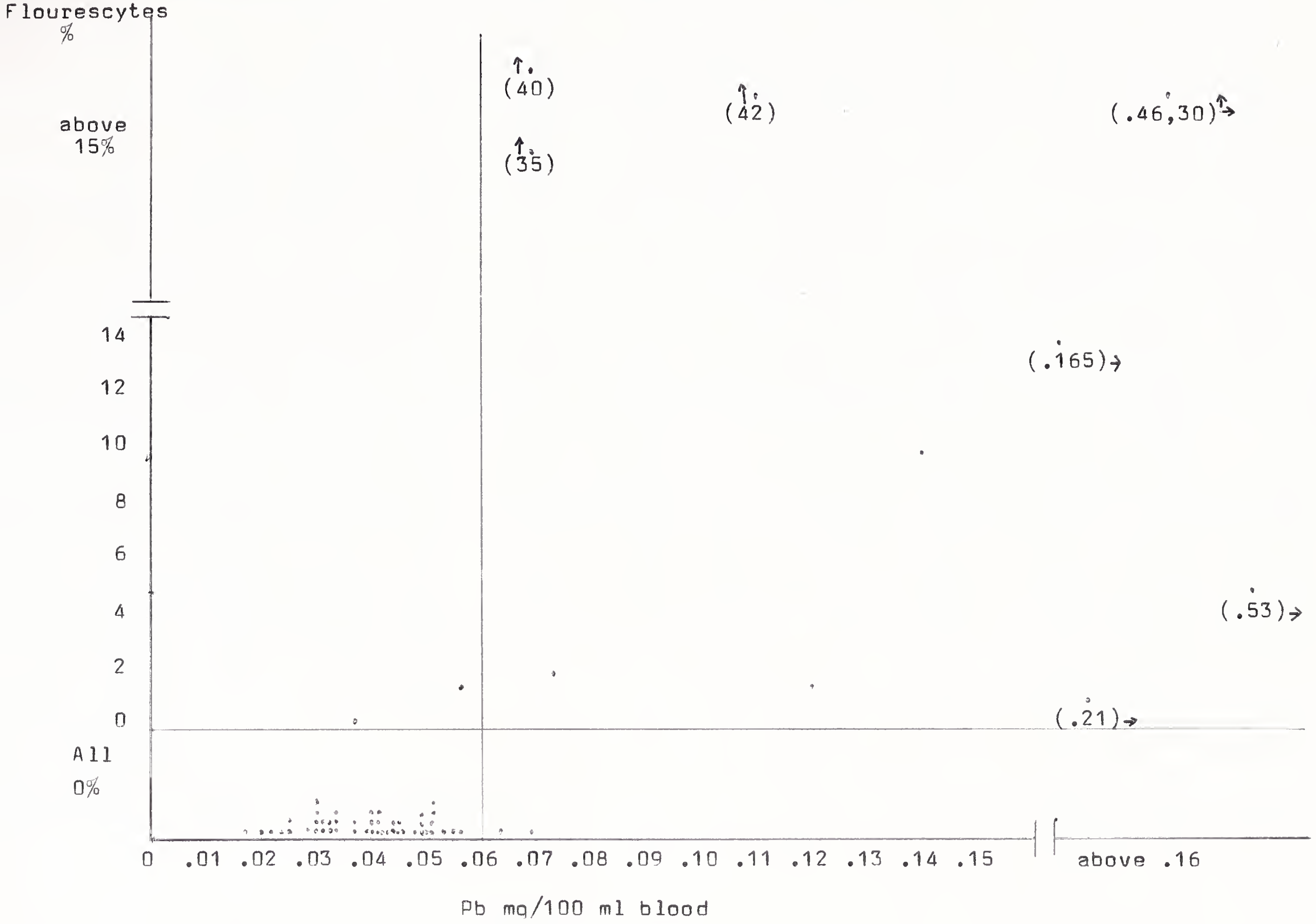

Flourescytes
%
above
15%
14
12
10
8
6
4
2
0
All
0%
(40)
(35)
(42)
(.46,30)
(.165)
(.53)
(.21)
0
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11
.12
.13
.14
.15
above .16
Pb mg/100 ml blood

Figure 7- Flourescytes vs ALA

The graph is of flourescytes in percent vs ALA in mg%.

1) Assuming ALA is the standard of true intoxication with abnormal values being those above 0.095 then the accuracy of flourescytes as a test is considered with the presence of any flourescence being abnormal.

Therefore:

with ALA greater than .095 -	flourescence present - 8 flourescence absent - 2
with ALA less than .095 -	flourescence present - 2 flourescence absent -34

Or:

True positives - 8
True negatives -34
False positives- 2
False negatives- 2

2) Now, using the same normal ranges as above, assume flourescytes to be the standard of intoxication and consider the accuracy of ALA as a test. One sees:

True positives - 8
True negatives -34
False positives- 2
False negatives- 2

Figure 7

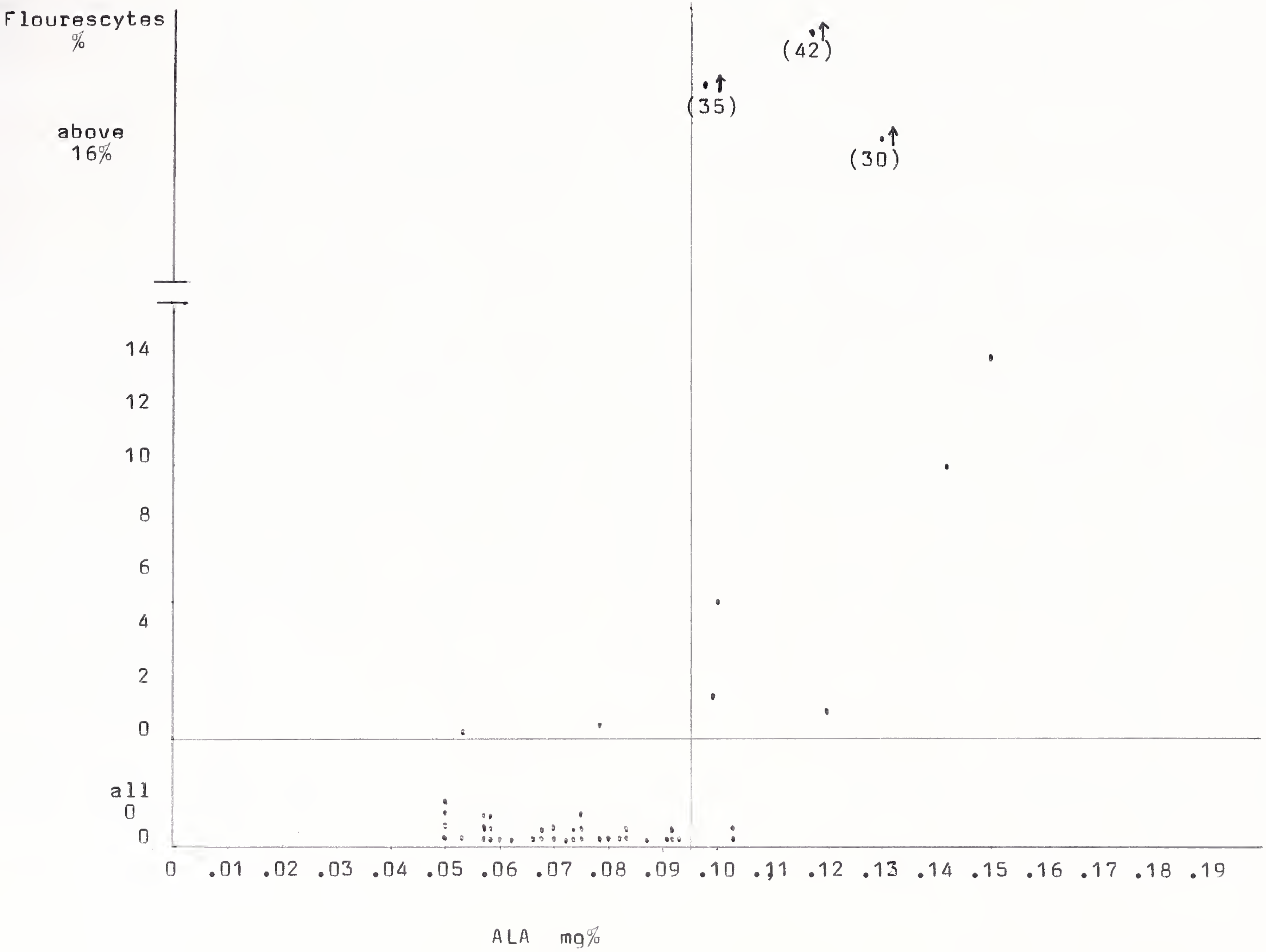

Flourescytes
%
above
16%
14
12
10
8
6
4
2
0
all
0
0
(35)
(42)
(30)
0
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11
.12
.13
.14
.15
.16
.17
.18
.19
ALA mg%

Figure 8, A&B- Basophilic stippling vs Pb, ALA

A) Basophilic stippling in % vs Pb in mg/100 ml blood

B) Basophilic stippling in % vs ALA in mg%

A) Assuming lead is the standard of intoxication with values above 0.06 as abnormals, the accuracy of basophilic stippling as a test is considered with any stippling as abnormal. Therefore:

with Pb greater than .06 -	stippling present - 3 stippling absent - 7
with Pb less than .06 -	stippling present - 0 stippling absent -40

Therefore there are:

True positives - 3
True negatives - 40
False positives- 0
False negatives- 7

B) Now with ALA as the standard with an abnormal range of greater than .095, basophilic stippling is again considered with the presence of any stippling being abnormal. One sees:

with ALA greater than .095 -	stippling present - 3 stippling absent - 5
with ALA less than .095 -	stippling present - 0 stippling absent -28

Or:

True positives - 3
True negatives - 28
False positives- 0
False negatives- 5

Figure 8, A&B

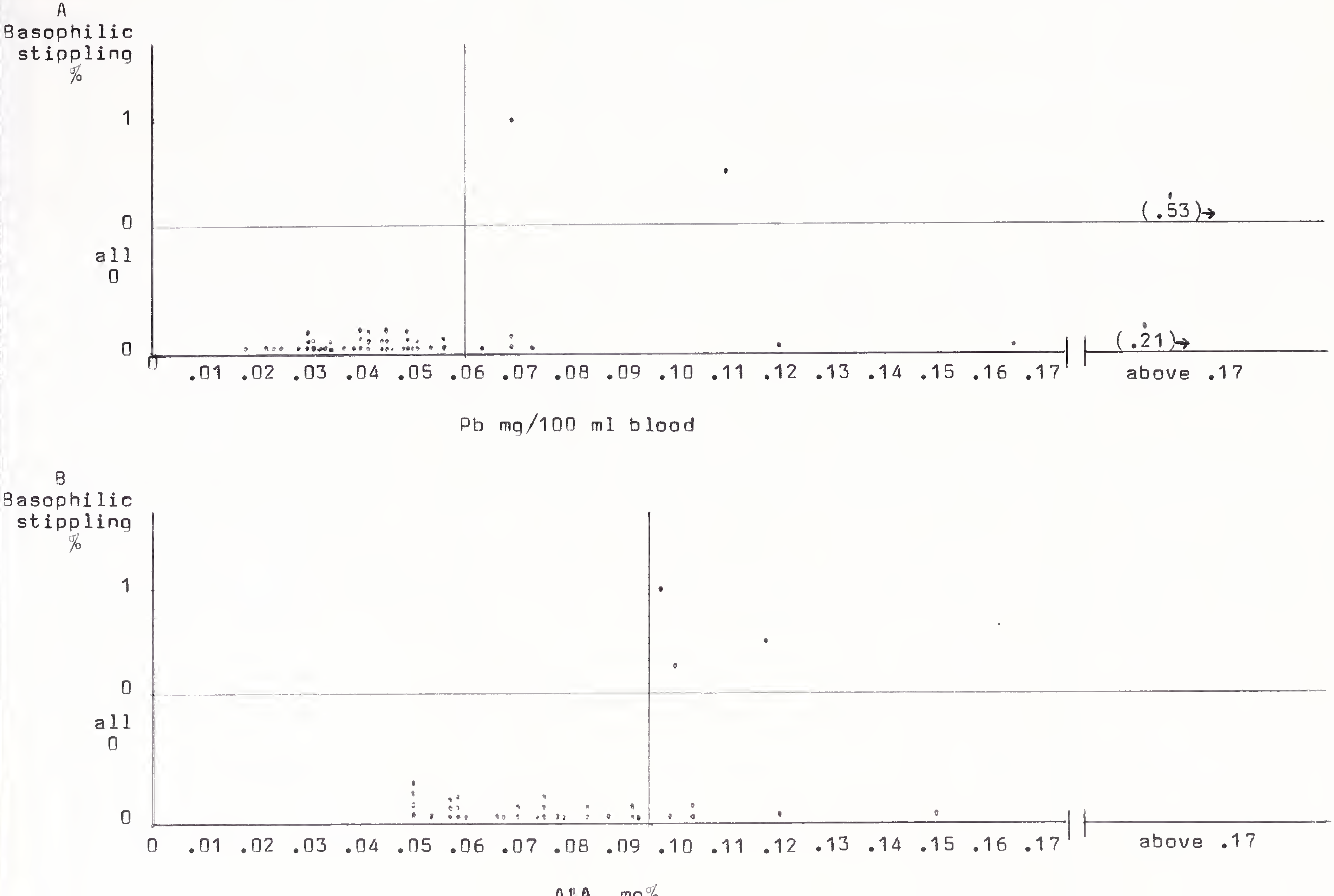

A
Basophilic stippling %
1
0
all 0
0
(.53)→
(.21)→
0
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11
.12
.13
.14
.15
.16
.17
above .17
Pb mg/100 ml blood
B
Basophilic stippling %
1
0
all 0
0
0
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11
.12
.13
.14
.15
.16
.17
above .17
ALA mg%

Figure 9, A&B- Pica vs Pb, ALA

A) Presence of history of pica vs Pb in mg/100 ml blood

B) Presence of history of pica vs ALA in mg%

A) Assuming Pb is the criterion for true intoxication with values above 0.06 being abnormal, then pica is present or absent in the following ranges:

with Pb greater than .06 - pica present - 10
pica absent - 0

with Pb less than .06 - pica present -25
pica absent -15

Or:

True positives - 10
True negatives - 15
False positives- 25
False negatives- 0

B) If ALA is the standard for true intoxication with ALA greater than 0.095 being abnormal, then pica is present or absent in the following ranges:

with ALA greater than .095 - pica present - 9
pica absent - 0

with ALA less than .095 - pica present -19
pica absent -10

Therefore:

True positives - 9
True negatives - 10
False positives- 19
False negatives- 0

Figure 9, A&B

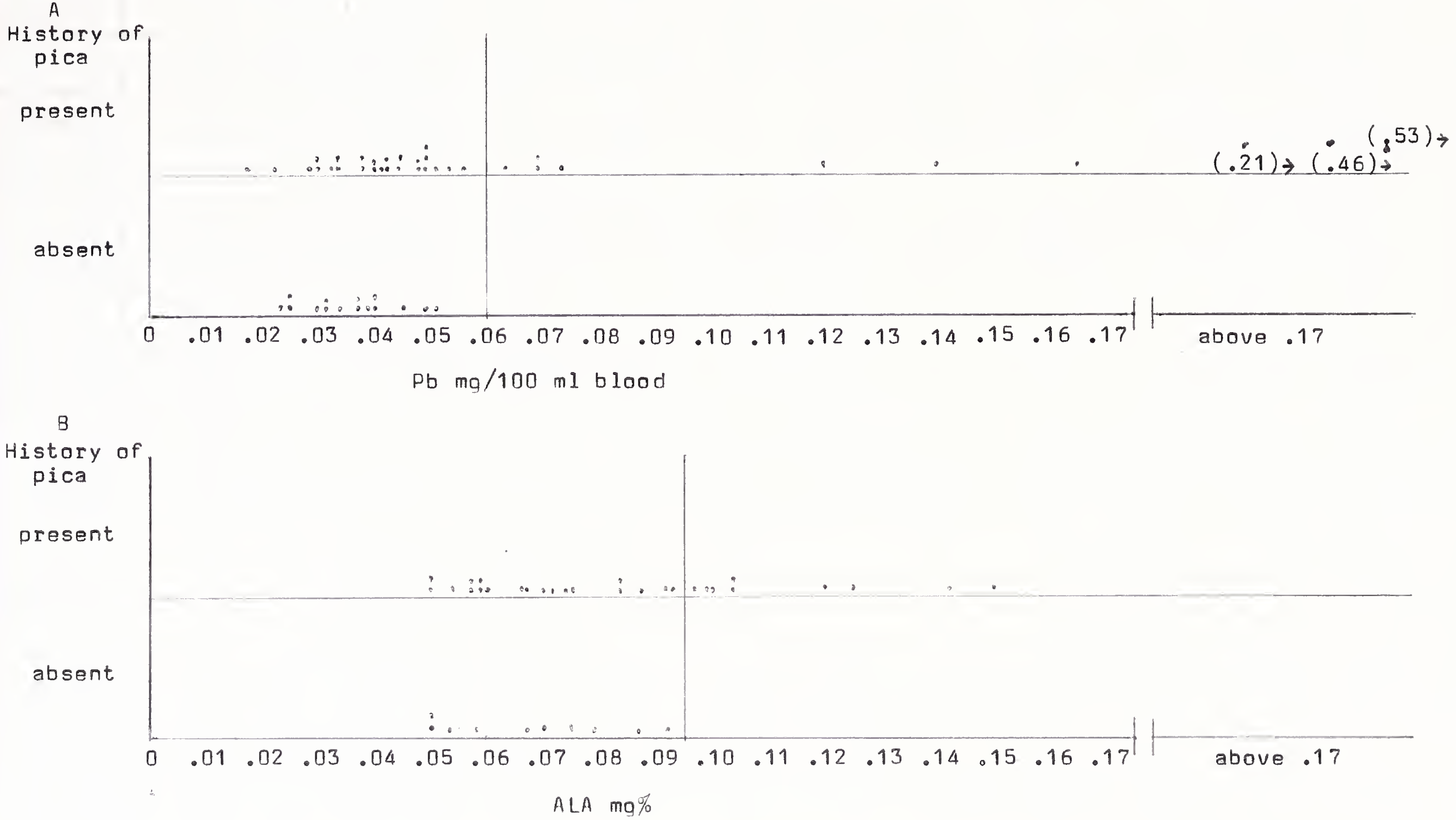

A
History of pica
present
absent
(.21)→
(.46)→
(.53)→
0
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11
.12
.13
.14
.15
.16
.17
above .17
Pb mg/100 ml blood
B
History of pica
present
absent
0
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11
.12
.13
.14
.15
.16
.17
above .17
ALA mg%

Figure 10, A&B- X-ray findings

A) X-rays of abdomen

B) X-rays of wrist

In both these section positive and negative X-rays are correlated with Pb, ALA, and flourescytes. Abnormal ranges are: Pb greater than 0.06 mg/100 ml blood; ALA greater than 0.095 mg%; flourescytes- any present.

A) X-ray of abdomen;	Positive	Negative
1) with Pb greater than .06 -	8	1
with Pb less than .06 -	5	7
2) with ALA greater than .095 -	9	1
with ALA less than .095 -	2	5
3) with flourescytes positive -	9	0
with flourescytes negative -	3	8

Therefore:	Pb	ALA	Flourescytes
True positives -	8	9	9
True negatives -	7	5	8
False positives-	5	2	3
False negatives-	1	1	0

B) X-ray of wrist:	Positive	Negative
1) with Pb greater than .06 -	1	1
with Pb less than .06 -	1	8
2) with ALA greater than .095 -	1	1
with ALA less than .095 -	1	5
3) with flourescytes present -	1	2
with flourescytes absent -	1	7

Therefore:	Pb	ALA	Flourescytes
True positives -	1	1	1
True negatives -	8	5	7
False positives-	1	1	1
False negatives-	1	1	2

Figure 10- X-ray findings

X-ray of abdomen					
Positive			Negative		
Pb	ALA	flour.	Pb	ALA	flour.
0.46	0.125	30%	0.069	0.103	0%
0.21	0.12	1%	0.051	0.073	0%
0.53	0.10	5%	0.042	-	0%
0.165	0.15	14%	0.022	-	0%
0.14	0.142	10%	0.034	0.078	0%
0.12	0.099	1.5%	0.031	0.053	0%
0.11	0.117	42%	0.033	0.083	0%
0.069	0.097	35%	0.03	0.083	0%
0.048	0.103	0%			
0.037	0.053	0.2%			
0.051	0.058	0%			
0.055	-	0%			
0.031	-	0%			

X-ray of wrist					
Positive			Negative		
Pb	ALA	flour.	Pb	ALA	flour.
0.46	0.125	30%	0.12	0.099	1.5%
0.034	0.078	0%	0.051	0.073	0%
			0.02	-	0%
			0.031	-	0%
			0.022	-	0%
			0.041	0.072	0%
			0.037	0.053	0.2%
			0.033	0.083	0%
			0.03	0.083	0%

Figure 11- Basophilic stippling vs flourescytes

Listed below is the comparison between basophilic stippling and flourescytes in the individual patients. Values are given in percents with abnormal being any flourescence or any basophilic stippling.

Stippled cells	Flourescytes	Number of pairs
1) positive	positive	3
1.0%	35%	
0.5%	42%	
0.25%	5%	
2) positive	negative	0
3) negative	positive	6
0	2%	
0	1.5%	
0	44%	
0	1.5%	
0	14%	
0	1%	
4) negative	negative	41

Figure 12- Cerebrospinal fluid vs Pb, ALA, flourescytes

Elevated CSF protein is taken to be greater than 40 mg%. Other abnormal values: Pb above 0.06 mg/100 ml blood; ALA above 0.095 mg%; flourescytes any present. Only 13 patients had lumbar punctures, only 2 of which had elevated proteins.

CSF proteins:	above 40	below 40
1) with Pb greater than .06 -	2	5
with Pb less than .06 -	0	5
2) with ALA greater than .095 -	2	7
with ALA less than .095 -	0	3
3) with flourescytes present -	2	5
with flourescytes absent -	0	3

Or simply: all those with abnormal CSF protein had all three parameters abnormal (Pb, ALA, flourescytes) but some patients with normal CSF values had one or more of the parameters in the abnormal range.

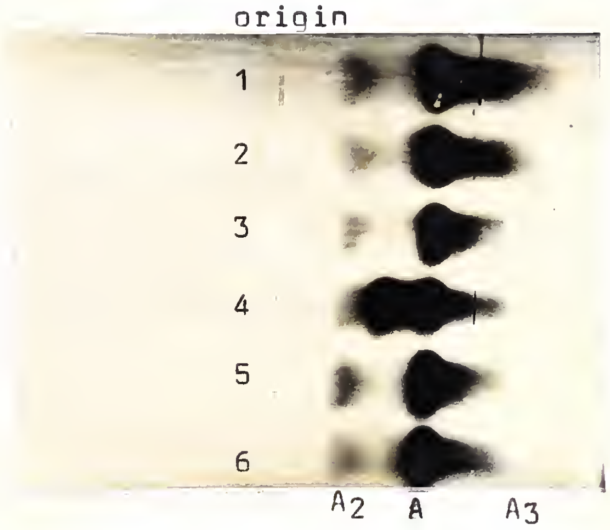
Figure 12

CSF protein	Pb	ALA	floures.
58	0.12	0.099	1.5%
42	0.11	0.117	42%
21	0.165	0.15	14%
21	0.14	0.142	10%
21	0.21	0.12	1%
17	0.53	0.10	5%
17	0.069	0.097	35%
15	0.069	0.103	0%
13	0.023	0.092	0%
20	0.042	-	0%
11	0.051	0.067	0%
8.5	0.053	0.057	0%
7	0.051	0.058	0%

Figure 13- Electrophoresis vs Pb, ALA, and flourescytes

Two patients had the electrophoretic patterns with Hb-Pb (Hb-A_3) as shown in Figure 11. In addition there were
8 patients with Pb greater than 0.06 with no Hb-Pb;
6 patients with ALA greater than 0.095 with no Hb-Pb; and
9 patients with positive flourescytes with no Hb-Pb.

Figure 13



Patients 1 and 2 have a zone that migrated beyond the major hemoglobin zone. This is the A_3 band and represents lead hemoglobin. Patient 4 had Hb-AC.

Laboratory values on patients 1 and 2:

#	A_1	A_2	A_3	Hgb total	Pb	ALA	Floures.
1	84%	3%	13%	7.8	.165	.15	14%
2	88%	2%	10%	7.4	.53	.10	5%

Discussion and Conclusions

As stated in the introduction, the purpose of this study is to determine which of the parameters investigated would be good for evaluation and/or screening of potential lead intoxicated patients and which parameters offer little to the evaluation of the lead intoxicated patients.

The three major parameters studied were blood lead, serum ALA, and flourescytes. The results were set up in such a manner that each of the above parameters could be considered the true measure of lead intoxication (although none probably exists) and the other parameters were then viewed in relation to the "true" parameter. The most important criteria for calling a given parameter a good test is that it minimizes the number of false negatives (those patients whose values are normal yet who are intoxicated) without including too many false positives(those patients with abnormal values yet who are normal). False positives are not a terrible problem if the test is inexpensive- that is a) if it is not overly time consuming for the tester or the patient and b) if it is not too costly financially. False negatives, however, are dangerous.

Other considerations for a good test are: 1) a test

that is not too complex and therefore not subject to procedural error; 2) a test that is reproducible within good limits; 3) a test that enables relatively easy attainment of sample material from the patient; 4) a test that can yield rapid results; 5) a test that is relatively specific for the condition being evaluated.

As was also stated in the introduction there was an insufficient number of lead intoxicated patients to draw definite conclusions. Therefore, an attempt will be made to make as many inferences as possible.

Lead, ALA, and flourescytes are used as the most specific criteria for lead intoxication since these most specifically measure the effect of lead ingestion a) by showing elevated levels of body lead through blood lead levels or b) by showing the effects of abnormal porphyrin metabolism in lead intoxication through ALA levels or the presence of flourescytes.

The sampling was bias by design. Only the potential lead intoxicated patients, defined in Materials and Methods, were studied. Therefore, the percent of positives of a certain variable does not reflect the number of positives that would be found in the population at large. With the knowledge that this is a biased study designed to look for lead intoxicated patients we found: 1) 66 patient samples were done; 2) of 62 who were tested for lead levels 14 (22%) were abnormal; 3) of 47 screened for ALA 11 (23%) were abnormal and 4) of 64 screened with

flourescytes 13 (20%) were positive. Hence, with a biased test approximately 20-25% were positive.

Before discussing the specific results and questions above a few general points will be considered. Later, the other parameters besides Pb, ALA, and flourescytes will be discussed and their relative worth will be considered.

Normal values:

The abnormal values used in this study were: Pb greater than 0.06 mg/100 ml blood; ALA greater than 0.095 mg %; flourescytes- any present. As explained earlier this value for lead is the most commonly accepted one. In looking at our graphs, Figure 4 for example, we see there would be little to gain by lowering this number and much to lose by raising it. In Figure 4 a drop of the Pb normal range to .05 would include no additional positive ALAs (that is would not eliminate false negatives) yet 6 additional false positives would be included. Similarly, lowering the ALA value to .08 would pick up one false negative lead but would also include 8 additional false positives. In Figure 6, a lowering of the normal Pb level to .05 would pick up one additional positive flourescyte along with 8 patients without them (false positives). Figure 7 shows, analagously, that lowering the ALA normal to ,08 would pick up no additional positive flourescytes (false negatives)

but would pick up 8 false positives. By similar arguments it is seen that raising the limits of Pb or ALA would serve to eliminate true positives, which is, obviously, unacceptable.

The normal for flourescence is very conveniently set at any flourescence. Since, as mentioned previously, quantification of flourescytes is both time consuming and difficult due to fading of flourescence there is little to be gained by doing it. Since it is being used only as a screening test one could rapidly look at a slide and if flourescence is present further evaluation could be done. Furthermore, by looking at Figures 6 and 7 it is seen that there does not appear to be a good direct correlation between ALAorPb and flourescytes- another argument against quantification. The last point will be discussed in greater detail later but suffice it to say that quantification of flourescytes is fairly difficult and relatively useless.

The normal values for ALA in the literature are: 0.019 mg%(Haeger-Aronsen, 11); 0.014 mg% (Feldman, 32); 0.0-56 mg% (Chisolm, 33). Our value was 0.095 mg%. In our standard curve, which was done from the beginning on six separate occassions, our slope was consistently 0.6 compared to Haeger-Aronsen's (11) slpoe of 1.06. Our normal controls were very reproducible with all values being within 5% of the mean. Hence, what error, if any, was

made was made consistently and our normal, though different from the published normals, was consistent. Therefore, our range will be used. One question that must be raised and that was not clearly answered in the literature, was how Haeger-Aronsen and Feldman read values in the range of 0.01 mg%. With the volumes of serum used (3 ml), with a standard ALA curve of ALA= O.D. x 1.06, and with a Beckman spectrophotometer, the values had to have been in the O.D. range of 0.01 which is a difficult range in which to read.

Pb vs ALA

Figure 5 considers these parameters against each other. It shows that when each is considered the "true" standard of lead intoxication the other gives 1 false negative and 1 false positive out of 11 possible positives. With lead as the standard the false negative with ALA (patient #65 Pb= .095, ALA= .083) the test for flourescytes was not done therefore we have no supporting evidence as to whether or not this is a true false negative. In the false negative with ALA as the standard (patient #25 Pb= .048 ALA= .103) there were no flourescytes found. This suggests that this may be a real negative, that is not a false negative, hence the lead value for this patient may have more validity. However, the number of cases is inconclusive to suggest one as a better parameter

hence it will be called a "draw".

Figure 4 correlates ALA vs Pb and shows that the probability of these two parameters measuring different things is small (less than .001). That is the probability is greater than 99.99% that they are measuring the same thing- lead intoxication.

Pb or ALA vs other parameters:

The remainder of Figure 4 gives correlations of variables not necessarily affected directly by ingestion but which are often changed in lead ingesters. The mean and standard deviation of the variables are also listed. Some of the variables, particularly lead, have a large standard deviation. It must be remembered that the standard deviation only measures the range of the variable, that is its spread about the mean. The large standard deviation of lead is due primarily to some of the very high values like .21, .46, .53. In the calculation of correlation coefficient the standard deviation is taken into account hence even with these large spread of values many of the correlations are still excellent.

The variables correlated first are Pb vs Hemoglobin, MCHC, % iron saturation of iron binding globulin, and reticulocytes, that is lead is the "standard". As can been seen there is a significant correltaion between Pb and Hgb, not unexpected in light of the effect of lead

on heme synthesis as shown previously in Figure 1. The correlation of Pb with % sat. is fair and this may reflect a coexistence of the diseases in a population prone to iron deficiency and lead intoxication. The other parameters, MCHC and retics, do not correlate particularly well. Next ALA is the "standard" against Hgb, MCHC, % sat, and retics. ALA correlates will against Hgb, as may be expected since both are intimately related to each other (Figure 1) and both are affected by lead. There is an unexpectedly good positive correlation with retics. This is interesting because on page 10 it was noted that lead intoxication results in increased reticulocytosis (4, 28). Since ALA gives a good correlation with reticulocytes one may surmise two events: 1) that the increased reticulocytosis occurs only after lead causes its toxic effects, that is disruption of heme synthesis or 2) that reticulocytes themselves are associated with a high ALA. Supposition 1 is definitely more likely as all patients with retics above 2% (patients# 15, 41, 57, 59) had both ALA and Pb elevated, while many other patients (# 19, 23, 25, 45, 56, 60, 63, 64) with elevated ALA and Pb had retics less than 1.5%. It seems that since ALA correlated well with % retics while Pb did not it appears that the reticulocytosis is probably an effect of Pb intoxication as is the level of ALA. Therefore, ALA is a better measure of lead effect than is the lead level alone.

In summary it is difficult to say whether ALA or Pb correlates best with the other parameters in Figure 4. Both correlate reasonably well with hemoglobin although lead does so to a better extent. Both correlate poorly with MCHC and % saturation. ALA correlates better with reticulocytes than does Pb as explained above. Therefore, ALA is probably a better indicator of the effect of lead than is the lead level itself, while the lead level is the better estimator of recent absorption.

Flourescytes:

Figures 6 and 7 correlate flourescytes against Pb and ALA. In figure 6 there were 12 abnormal leads, 2 of which had no flourescence or there were 2 of 12 (16%) false negatives. Comparing flourescytes vs ALA in Figure 7 it can be seen that of the 10 positive ALAs 2, or 20%, had false negatives. Therefore, if flourescence was a screening test 16-20% of the patients would have false negatives, that is, they would be called normal while having either ALA or Pb abnormal.

There were also 2 false positives in each case, thereby giving 16-20% of the patients with positive flourescytes while having negative Pb or ALA. Hence, about one-fifth of the patients would be needlessly worked up.

Further examination of the false negatives show: the 4 false negative points actually represent three

patients (# 25,41,42). In these3 patients # 41 has both Pb and ALA abnormal and is, therefore, a real false negative. Patient # 25 had an abnormal ALA and a normal Pb and is only a false negative against one parameter. Patient # 42 had an abnormal lead and no ALA done so it, too, is a false negative against one criterion. Of note is that all the abnormal values in these false negatives are close to the normal ranges (# 25- ALA= .103, Pb= .048; # 41- Pb= .069, ALA= .103; # 42- Pb= .063). It can then be seen that the false negatives do exist but they are in the ALA and Pb range that is just above the normal limit.

In summary, then, there is a definite 16-20% risk of false negatives using flourescytes as a screen. However, none of these come in severely intoxicated patients, that is those with very high leads or ALAs. Again, a larger sample would help but we can imply that flourescytes can be used as a screen but a definite risk is involved. Serial measurements were not done and it would be interesting to see whether or not these children would have developed positive flourescytes later.

Another interesting point is suggested by looking at the distribution of points in Figures 6 and 7. It seems as if the number of flourescytes goes up as the ALA value is higher. Most of the patients with significantly high ALAs have a high percentage of flourescytes.

On the contrary, with lead there are some very high values with only a small percent of flourescytes. These observations are admittedly just casual and,as noted, quantification is difficult. However, if it is as it appears, the ALA correlates better with flourescytes than does lead, which is understandable as both ALA and flourescytes measure the effect of lead on heme synthesis.

Basophilic stippling:

Figure 8 shows the occurrence of basophilic stippling in patients with elevated Pb and elevated ALA. Stippled cells are seen in 3 of 7 or 30% of patients with elevated lead and 3 of 5 or 38% of patients with elevated ALA. The three patients with basophilic stippling have both ALA and Pb elevated. The percentage of intoxicated patients having basophilic stippling in our study is similar to the 30% (5) to 60% (6,32,36) found in the literature.

Figure 11 shows the comparative occurrence of flourescytes and basophilic stippled red blood cells. The data here show that whenever there is basophilic stippling there is also flourescence; there is never basophilic stippling without flourescence; and flourescence occurs when stippling does not. Therefore, if flourescytes are screened for there seems to be no need to look for basophilic stippling.

Our information suggests what was suggested previously, that is, that the finding of basophilic stippled cells should arouse suspicion of lead intoxication and that further studies should be done (34). With 40-70% false negatives it is certainly not a useful test. Although there are no false positives here, basophilic stippled cells are also found in other situations (27,31) and are not specific for lead intoxication.

Pica:

Figure 9 shows the occurrence of pica in our study. The finding of pica in about 70% of our patients is due to the bias of the study which specifically screened patients with pica. It can be seen that with either ALA or Pb there were no patients with abnormal values that did not have pica, and about 60% of those with normal values had pica. These results agree with the literature in that 10-30% (1,4,17) of patients with pica have evidence of lead intoxication. In addition we showed that all patients with intoxication had pica- a not unexpected finding.

X-ray:

The X-ray results are in Figure 10. The value of doing abdominal or bone X-rays is questioned, first because radiation is dangerous in young children,and second because the results of the X-ray are not particularly

useful. The abdominal X-ray will be discussed first. In this group all but one of the patients with negative Pb or ALA had a negative X-ray thereby giving the patients needless radiation when blood tests gave the same result. The one false negative is obviously of no value. Of the positive X-rays 40% were false positives with lead and 22% were false positives with ALA. Since two of the false positives with Pb did not have ALAs done there is an excellent chance that these would have had normal ALA values thereby raising the ALA false positives to 44%. Therefore, almost half of the patients with positive abdominal films did not have evidence of intoxication so blood tests would have to be done to prove whether or not the patient was intoxicated. It can thus be seen that a patient should neither be treated for a positive X-ray nor not treated for a negative one, hence, this seems to be a needless test. The results of the wrist X-rays are from a smaller population but they appear to have as little use as do the abdominal films. Here, especially, there are so many patients that were true negatives (63-73%) that could have been spared the radiation and had a simple blood test instead. Half of the two positives were false positives, hence even a positive wrist X-ray needs chemical verification. We, then, agree with Sartain's conclusions (51) mentioned previously: that x-ray findings should be a secondary consideration and

that the presence of x-ray findings should not presume intoxication nor should their absence preclude it. Taking this one step further there seems to be no need or use in doing x-rays at all.

CSF:

Figure 12, which compares CSF vs Pb, ALA, and flourescytes, suggests that one cannot tell if central nervous system damage has occurred (as measured by CSF protein) by the presence of abnormal values of any of the three major parameters. The two patients who had elevated CSF protein had all 3 parameters in the abnormal range, yet 5 other patients with all 3 parameters abnormal had normal CSF protein.

Hb-Pb:

Figure 13 shows a copy of one of the electrophoresis patterns that included the only two patients that had Hb-Pb. As can be seen both patients had abnormal Pb, ALA, and flourescyte values. There were, however, many patients with abnormal parameters that did not have Hb-Pb. Presence of Hb-Pb is an interesting phenomenon of which observers should be aware when interpreting electrophoretic patterns of anemic children who may have plumbism. It has little use as a screening device due to the time involved, and to the many false negatives present.

Urine:

There is little to be gained by urine screening. Patients with severe lead intoxication shoul have urine protein and glucose measured as a baseline, and this is usually done when they are admitted to the hospital. There seems to be little to be gained by assaying urinary aminoacids as there were no positives in the severely intoxicated patients who were tested.

Uric acid:

None of the patients had elevated uric acids. Since this is probably a phenomenon of chronic ingestion (44,45,46) this result is not unexpected.

Conclusions:

Some final conclusions about the different possible screening tests will now be drawn. Lead vs ALA vs flourescytes will be discussed here and all the final conclusions will be listed in the Summary.

The comparison of ALA vs Pb as the "true" parameter showed a "draw", that is the same number of false negatives (8%) occurs when each is tested against the other. From comparison of ALA and Pb to hemoglobin, reticulocytes and flourescytes it appears that the Pb level is a better measurement of recent ingestion while ALA is a better measurement of the toxic effects of lead.

The flourescytes gave approximately the same

number of false negatives when used as a screen with lead as the standard (2 of 12 or 16% false negatives) and with ALA as the standard (2 of 10 or 20%). These, however, occurred in lead and ALA ranges just outside the normal range. There is, thus, a slightly better chance of having false negatives using flourescytes as a screen than if either ALA or Pb is used to screen the children.

Consideration will now be given as to which of the three is a "good test" according to the criteria listed earlier in the discussion: 1)complexity-Lead requires 12 ml of blood and at least 2 days before results return since the blood must be sent to the state lab. ALA requires only 5ml of blood (2-3 ml of plasma). The test itself has many steps and it takes about 2 hours to assay ALA in 6 patients and a control. The results, however, are attainable within hours. Flourescytes would require only a capillary tube of blood making only a fingerstick necessary rather than the venipuncture required for Pb and ALA. It is a simple test to do once one learns how to use a UV scope and a sample can be screened in a matter of minutes. The major problems here are in the availability of a UV scope and in the fact that once the scope is turned off it must remain off for 2 hours. 2)reproducibility- Pb and ALA are reproducible to within reasonable limits- Pb +/- 0.01 mg, ALA +/- 5%.

All samples from a tube of blood will give flourescence if it is present in that patient. 3) sampling- As suggested in 1 it is easy to get samples for flourescytes as only a fingerstick is needed. ALA and Pb require venipunctures with Pb needing 12 ml. 4) rapidity of results- Flourescyte screening requires a few minutes to a few hours depending on the availability of the UV scope. ALA can be done within hours; Pb requires days. 5) specificity- Lead levels reflect ingestion of lead; ALA and flourescytes reflecr the effects of lead.

All samples were [illegible] if it is [illegible] as in [illegible] only a [illegible] puncture will [illegible] Tobramycin [illegible] hours depending on the analyzer [illegible] etc can be done [illegible] specifications [illegible] and fluorescence [illegible].

Summary

It is impossible to categorically call one test the best screening test. The evidence will be summarized below so the reader can be aware of the advantages and risks of each.

1) 66 patients were screened for lead intoxication. On the basis of a bias selection 20-25% had evidence of lead intoxication.
2) Neither ALA nor lead has a particular advantage over the other as far as the number of false negatives is concerned.
3) Flourescytes has slightly more false negatives than ALA or Pb. These false negatives occur in ranges of ALA and Pb that are just above the normal.
4) Pb levels appear to reflect recently ingested lead. ALA and flourescytes reflect the effects of lead.
5) Flourescytes is the easiest test to do and it yields rapid results. ALA is fairly rigorous to do but yields results in the same day. Pb is done by the the state lab but two or more days are required for results. All are reasonably reproducible.
6) Pica- 30% of the patients with pica had evidence of lead intoxication.
7) Hemoglobin, hematocrit, indices- These are not specific but may indicate patients that should be screened.

8) Basophilic stippling- This is not specific and is not worth looking for as the other parameters pick up the same patients. If it is found on a routine smear more definitive tests should be done.

9) Iron, iron binding capacity, % saturation- These add little to the evaluation of lead intoxication but may indicate a concommitant iron deficiency.

10) Urine- Baseline protein and glucose determinations on patients admitted for intoxication would be useful. Aminoacid screening is time consuming, costly, and not helpful.

11) Uric acid- All the values were normal. Since it is elevated only in chronic ingestion it is not worth doing in children.

12) Cerebrospinal fluid- Lumbar punctures should be done in the presence of severe intoxication especially when signs and symptoms of CNS damage are present. The ALA, Pb and flourescyte values do not appear to be of help in choosing who to tap.

13) Hemoglobin electrophoresis- In screening children for causes of anemia one should be aware of the fact that a Hb-A_3 (Hb-Pb) band may appear in lead intoxicated patients. It is an interesting finding but probably not worth looking for routinely in lead poisoned patients.

14) X-rays of the abdomen and long bones- Neither positive nor negative x-rays aid in the evaluation or management of patients. Since there is a hazard to radiation x-rays should be omitted.

Appendix 1- "raw" data

Patient	Age months	Hgb	Hct	MCHC	Retics	Basoph stippl	Fe	IBC	% sat
1	91	13.0	37	35	-	-	-	-	-
2	17	-	-	-	-	-	67	493	12
3	40	-	-	-	-	-	40	346	12
4	45	13.0	36	35	0.2	-	87	394	22
5	25	11.6	37	31	-	-	77	407	19
6	57	12.5	37	31	0.1	-	63	452	14
7	22	11.4	35	32	1.2	-	65	446	15
8	34	11.6	38	30	1.9	0	70	356	20
9	17	12.2	38	32	1.0	0	60	409	15
10	48	-	-	-	-	-	120	280	43
11	32	11.4	38	30	1.2	0	106	467	22
12	31	12.2	37	33	0.5	0	51	443	12
13	22	12.4	38	33	1.4	0	40	388	10
14	34	7.8	30	26	-	-	28	516	5
15	31	10.3	35	30	3.0	1	57	492	12
16	19	12.0	38	32	0.4	0	81	363	22
17	16	10.9	37	29	0.4	0	61	461	13
18	23	12.0	40	30	0.8	0	63	511	12
19	40	10.0	34	31	1.5	0	-	-	-
20	66	12.9	42	31	0.5	0	51	472	11
21	49	12.9	42	31	1.0	0	58	341	17
22	28	10.0	36	28	1.7	0	-	-	-
23	47	12.0	36	36	1.6	-	-	-	-
24	33	13.5	39	35	1.0	0	115	314	38

Patient	Lead at Hct 35	ALA	Flour.	Uric acid	Pica	X-ray		Misc.
						abd	wrist	
1	.025	.087	0	-	no			
2	-	.078	0.5	-				
3	-	-	0	-		neg	neg	Hb-AC
4	.051	.073	0	-		neg	neg	
5	.02	-	0	-				
6	.055	-	0	-		pos	neg	
7	.042	-	0	-	yes	neg	-	nl CSF
8	.073	-	2	3.3	yes			
9	.031	-	0	2.7	no	pos	neg	Hb-AS
10	-	.062	0	4.1				
11	.039	-	0	6.0	no			
12	.04	-	0	4.5	yes			
13	.049	-	0	-	yes			
14	.037	.067	0	4.1	yes			
15	.069	.097	35	-	yes	pos	-	nl CSF
16	.044	.057	0	3.4	yes			
17	.04	.093	0	4.0	yes			
18	.037	-	0	3.4	yes			
19	.049	.092	0	-	yes			
20	.022	-	0	3.4	yes	neg	neg	
21	.044	.075	0	3.5				
22	.041	-	0	-	no			
23	.061	.11	-	-				
24	.033	.074	0	5.2	yes			Hb-AC

Patient	Age months	Hgb	Hct	MCHC	Retics	Basoph stippl	Fe	IBC	% sat
25	25	10.0	35	29	1.2	0	51	351	10
26	30	10.3	36	29	0.9	0	43	543	8
27	14	9.0	33	27	1.4	0	16	555	3
28	13	8.8	33	27	2.5	0	-	-	-
29	9	7.0	29	25	0.9	0	27	636	4
30	33	13.7	38	36	0.5	0	-	-	-
31	50	12.2	38	32	2.0	0	134	450	30
32	14	11.6	37	31	1.6	0	88	390	23
33	47	12.0	38	32	0.8	0	87	264	33
34	25	10.3	35	29	1.8	0	21	629	3
35	"	12.0	35	34	0.8	-	66	409	16
36	32	12.6	39	32	1.0	0	56	346	16
37	32	12.2	37	33	1.4	0	77	367	21
38	20	11.1	37	30	0.4	0	66	439	15
39	23	9.4	33	28	0.7	0	-	-	-
40	23	12.4	36	34	0.8	0	-	-	-
41	24	14.2	40	35	2.6	0	-	-	-
42	"	12.2	41	30	1.2	0	65	438	15
43	29	7.8	23	34	0.5	0.5	116	466	25
44	"	10.0	35	29	1.4	0	-	-	-
45	25	10.5	36	29	1.2	0	-	-	-
46	26	11.8	33	36	1.0	0	50	396	13
47	73	11.1	37	30	0.4	0	69	375	18
48	44	12.0	37	32	0.6	0	60	432	14

Patient	Lead at Hct 35	ALA	Flour.	Uric acid	Pica	X-ray abd	X-ray wrist	Misc.
25	.048	.103	0	3.4	yes	pos		
26	.041	.072	0	4.8	yes	pos	neg	
27	.056	-	1.5	-				
28	.028	.050	0	-	yes			
29	.051	.067	0	5.8	no			nl CSF
30	.034	.058	0	-	yes			Hb-AS
31	.034	.078	0	5.5	no	neg	pos	
32	.03	.06	0	3.7	no			Hb-AS
33	.031	.053	0	5.2	no	neg		
34	.048	.087	0	-	yes			Hb-AS
35	.037	.053	0.2	5.4	yes	pos	neg	Hb-AS
36	.033	.083	0	2.8	yes	neg	neg	
37	.03	.083	0	3.8	yes	neg	neg	
38	.025	.050	0	5.4	no			
39	.053	.057	0	-	yes			nl CSF Hb-AS
40	.041	-	0	4.2	yes			
41	.069	.103	0	-	yes	neg		nl CSF
42	.063	-	0	-	yes			
43	.11	.117	42	2.5		pos		CSF prot = 42
44	.069	-	40	-				
45	.051	.058	0	-	yes	pos		nl CSF Hb-AS
46	.056	.066	0	2.8	yes			Hb-AC
47	.04	.07	0	3.0	no			
48	.03	.05	0	4.3	yes			

Patient	Age months	Hgb	Hct	MCHC	Retic	Basoph stippl	Fe	IBC	% sat
49	58	10.5	34	31	1.0	0	34	326	10
50	69	12.0	37	32	0.8	0	27	382	7
51	30	14.4	43	33	0.6	0	96	473	20
52	37	10.0	32	31	1.4	0	66	473	14
53	30	11.4	37	31	1.6	0	39	496	8
54	50	8.6	33	26	1.0	0	28	600	5
55	21	-	-	-	0.6	0	28	569	5
56	51	14.6	41	28	1.0	0	46	303	15
57	18	9.0	29	31	2.0	0	48	436	11
58	32	7.4	25	30	1.0	0.25	24	571	4
59	38	7.8	27	29	2.5	0	25	507	5
60	21	9.8	33	30	1.8	0	35	495	7
61	15	9.5	31	31	-	-	-	-	-
62	10	7.9	29	27	-	-	-	-	-
63	15	9.8	32	31	1.0	0	34	486	7
64	43	13.2	36	37	1.2	-	41	308	13
65	24	8.0	27	30	1.2	-	19	560	4
66	24	12.0	35	35	-	-	66	384	17

Patient	Lead	ALA	Flour.	Uric	Pica	X-ray		Misc.
	at Hct 35			acid		abd	wrist	
49	.049	-	0	3.0				
50	.05	.058	0	5.3				
51	.017	-	0	3.5	yes			
52	.046	.057	0	4.1	no			Hb-AC
53	.03	.075	0	2.8	no			
54	.045	.05	0	3.2	no			
55	-	.07	0	2.1	yes			
56	.045	.075	0	3.9	yes			
57	.12	.099	1.5	3.8	yes	pos	neg	CSF prot = 58
58	.53	.10	5	2.1	yes	pos		nl CSF urine tr. prot Hb-A$_3$
59	.165	.15	14	3.1	yes	pos		nl CSF Hb-A$_3$
60	.21	.12	1	3.6	yes	pos		nl CSF urine 1+ gluc
61	.14	.142	10	4.7	yes	pos		nl CSF
62	.46	.125	30	3.6	yes	pos	pos	urine tr prot 1+ gluc
63	.023	.092	0	3.5	no			nl CSF
64	.051	.091	0	3.9				
65	.095	.083	-	3.9				
66	.032	-	0	4.4	no			

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