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Evolution of the Management and Prevention of Childhood Lead Poisoning: Dependence of Advances in Public Health on Technological Advances in the Determination of Lead and Related Biochemical Indicators of Its Toxicity

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INTRODUCTION

Childhood lead poisoning was first reported from Brisbane, Australia (Gibson *et al.*, 1892) in 1892, although lead poisoning in adults had been described for many centuries before that. Childhood lead poisoning (due to ingestion of deteriorating paint) was first reported in the United States from the Johns Hopkins Hospital in 1914 (Thomas and Blackfan, 1914). Since that time progress in the United States may be divided technologically into (1) pre-dithizone era, (2) dithizone era, and (3) atomic absorption (and electrochemical) era. These advances will be reviewed step by step to show how each technological advance facilitated improvement in recognition, diagnosis, and public health steps in the prevention of childhood lead poisoning.

PRE-DITHIZONE ERA

In 1892, Gibson *et al.* reported on 10 cases of plumbism carefully characterized by electrical studies of the distribution of lead palsy in children, which, they stated, differed from its distribution in adults. They also noted that such cases had previously occurred but had not been carefully documented. The children were treated orally with potassium iodide and according to them, made a "complete recovery" in 1–8 months. Gibson *et al.* (1892) looked for the source, but were unable to document any uniform source, although they did trace two of their cases to children who chewed on lead foil used to wrap candies. Later they suspected water, but this was not confirmed, and then paint. In fact, Gibson (1904) made a plea for painted railings and walls in the houses, as the main source of the lead in childhood lead poisoning.

In 1978 the Royal Children's Hospital in Brisbane was celebrating its 100th anniversary. Fison (1978) cited J. Lockhart Gibson as one of the hospital's four physicians who contributed most to its accomplishments during the first century of the hospital's existence. Fison summarized the professional career of J. Lockhart Gibson as follows:

> Toward the end of last century and in the first quarter of this one, chronic lead poisoning was prevalent in Queensland children, causing weight loss, irritability, abdominal pain, peripheral neuritis with characteristic wrist drop, increased intracranial pressure and nephrosclerosis. Suspected by Dr. Turner, specimens of tank water were analyzed by the Government Analyst in 1896, and shown to contain lead. Later the real cause of the trouble was found by J. Lockhart Gibson, and ophthalmic surgeon, to be contamination of children's hands by weathering lead paint. Gibson conducted a vigorous campaign against the use of lead paints, opposed by many vested interests, and eventually had them banned by legislation from use in any situation where children could be exposed to them. At the time southern physicians were skeptical, for the condition seemed to stop abruptly at the Queensland border, and they regarded the condition as a delusion held by their despised colleagues in that primitive northern State. The difference, however, lay in the housing—Queensland houses, especially in those days, had large verandahs where children played, particularly during summer rains. Verandah paints weather quickly and playing children would soon have their hands coated with powdery lead sulfate, inevitably carried to their mouth and digestive tracts. The ban on lead paints has long since become

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universal so that, thanks to J. Lockhart Gibson, plumbism in children is now a rare condition.

It is probable that Queensland, Australia, adhered to the international convention (Markowitz and Rosner, 2000) in 1922 (Council of the Queensland Branch BMA) of banning the use of lead paint where children could reach it.[1]

Markowitz and Rosner have presented in considerable detail the role of the lead industries between 1900 and 1955 in waging an aggressive marketing and public information campaign to persuade the public that lead paint was appropriate for indoor use. They downplayed the hazards of lead paint to children, although as we have seen it had been well documented. Markowitz and Rosner note that the danger of lead paint poisoning was well known outside of the United States and indeed they note that: "many countries enacting bans or restrictions on the use of white lead for interior paint. France, Belgium, and Austria in 1909; Tunisia and Greece in 1922; Czechoslovakia in 1924; Great Britain, Sweden, and Belgium in 1926; Poland in 1927; Spain and Yugoslavia in 1931; and Cuba in 1934. In 1922 the Third International Labor Conference of the League of Nations recommended the banning of white lead for interior use. In the United States and Canada, there were calls for the use of non-lead-based paints in interiors." Finally the United States banned in 1978 the transport in interstate commerce of lead-based paints intended for residential use containing 0.06% or more of lead (NRC, 1976).

Although the earlier writers were preoccupied by detailed descriptions of the clinical features of childhood lead poisoning and the search for the source, United States workers often made a comment that those who survived a bout of acute encephalopathy made a complete recovery (McKhann, 1926). It is clear that "complete recovery" meant the absence of acute manifestations. The failure to initiate follow-up of symptomatic cases was no doubt due to the total dependence on physicians on identifying the symptoms and signs of childhood lead poisoning and the absence of any feasible laboratory procedure such as the dithizone procedure, with which lead could be measured in blood, urine, and tissues to document the clinical diagnoses. It is of note that Levinson and Harris (1936) did call attention to the fact that children should perhaps be followed long term for neurobehavioral disturbances. Not until 1943 were sequelae well documented by Byers and Lord, who studied 20 children who had symptomatic lead poisoning in early childhood, and were followed to school age. They found that 19 of the 20 that they followed were excluded from first and second grade despite the presence of normal IQ values and mainly because of aggressive antisocial, uncontrollable behavior.

During the great depression in the United States an epidemic of childhood lead poisoning occurred in Baltimore. The description of this epidemic is a classic in epidemiology (Williams *et al.*, 1933). In 1931 a child was admitted to the inpatient pediatric service at the Johns Hopkins Hospital with a tentative diagnosis of tuberculous meningitis. The intern seeing this case doubted the diagnosis. She took the unusual step, for interns, of going to the child's home to see if she could discover the cause. There she met one Melrose Easter, a drunken man who aspired to be a physician. He opined to her that it was those things, because they smelled so bad, and pointed to lead acid battery casings. Upon return she found that the child's findings were consistent with lead poisoning. Dr. Park, chief of Pediatrics, and staff did one other important thing; they reported the matter to the Health Department. Dr. Huntington Williams,[2] the newly appointed Health Commissioner (1931–1962), dispatched public health officials to investigate.

They discovered that six junk dealers were distributing these battery casings to the poor as a good-will gesture. The health department had the casings picked up. The problem was that physicians, having recognized acute lead poisoning, continued to recognize the disease after the offending battery casings were removed. The cases were documented because a National Research Council fellow at the Johns Hopkins School of Hygiene could operate an emission spectrograph (Blumberg and Scott, 1935). The spectrograph had been a gift to the Johns Hopkins School of Public Health. Dr. Park, Chief of Pediatrics, at Johns Hopkins has depended

[1] The author visited Australia in 1978 and saw the few remaining colonial houses that were responsible for lead paint poisoning in Queensland. He also was presented with cases of childhood lead encephalopathy in the states of New South Wales (Sydney) and Victoria (Melbourne). In 1968 the author met Brian Emmerson (1963a, b, 1973), who had been studying lead's renal sequelae. Dr. Emmerson was carrying out these studies in Brisbane and noted that even in the 1960s, when the public health nurse delivered the birth certificate for a new infant, she also took samples of paint to be tested for lead. If found, it had to be removed.

[2] Dr. Huntington Williams' professional career was elegantly described by Dr. Elizabeth Fee (1990). He was a pioneer in the public health approach to childhood lead poisoning and, indeed, was the early driving force in this endeavor.

upon him, when the diagnosis of lead poisoning came up. Blumberg left Johns Hopkins and there was no one to operate the emission spectrograph in his place.

DITHIZONE ERA

Dr. Park approached the Health Department. They could not afford a spectrograph and so a newly employed toxicologist, Dr. Emanuel Kaplan who had recently received his Sc.D. in chemistry at Johns Hopkins University, was dispatched to DuPont in Wilmington, Delaware, to learn the new dithizone technique. This was developed in Germany and had just been introduced in this country. He returned and the Health Department set up a free diagnostic blood lead laboratory. It was primarily based on the method of Wilkins *et al.* (1935) with modifications including the technique of Clifford and Wichmann (1936). This is a very exacting technique, which requires the utmost attention to detail. The pitfalls in the test have been more recently described in Airborne Lead in Perspective (NRC, 1972). With this technique the City Health Department laboratory could initially examine 6 specimens at a time. This required an outlay over a period of 4 years of somewhat more than $400. The cost of the dithizone testing was about 25 cents per sample (Kaplan and McDonald, 1942). With this technique in place the Baltimore City Health Department (BCHD) set up a free diagnostic blood lead laboratory, in 1935, which operated until the laboratory was transferred to the State in the 1960s, where it still operates. During the past few years it has not been free.

It may be said that Dr. Huntington Williams, Commissioner of Health of the city of Baltimore, was the first to recognize and act upon childhood lead poisoning as a public health issue. The Department of Health was a pioneer and virtually alone in this endeavor until the 1960s. The laboratory provided lead-free blood lead collection kits and actually would accept samples only in these tubes, which had been soaked in nitric acid and then rinsed with lead-free water and dried. For collection of adult blood they supplied sterile lead-free 19-gauge needles. When the author began his studies in 1952, his lead laboratory prepared syringes and needles to use in children at the Johns Hopkins and Baltimore City Hospitals. This was continued until polyethylene syringes and stainless-steel butterflies became commercially available in the 1960s.

In 1942 McDonald and Kaplan reported their experience between 1931 and 1940. They noted that among children there were 49 fatal cases of lead poisoning and 35 nonfatal cases. Among adults, during the same time period, there were 5 fatalities and 72 nonfatal cases. During the early years BCHD laboratory was the only laboratory in Baltimore doing blood lead determinations, except in relation to chelation therapy during the 1950s and thereafter. During the period 1931–1940 in the entire United States there were 200 deaths reported from lead poisoning in children less than 15 years of age. Forty-nine or 24.3% of the total were reported from the city of Baltimore, which at the time constituted only 0.65% of the total child population of the United States..

In 1952, Williams *et al.* (1952) summarized the Baltimore experience during the preceding 20 years. This report contains interesting facets including recognition of the fact that there is a seasonal distribution of childhood lead poisoning. Eighty-five percent of the cases occurred during the warmer months and virtually no new cases toward the end of the winter of each year. It was also recognized that deteriorating paint was the principal source of lead. The Health Department used traditional public health methods by attempting to educate the people to keep their children from chewing on paint and through publicity, a policy that has never worked. A public health nurse was assigned to work with all cases of recognized lead poisoning. That continues to be the policy even today.

In the 1950s the author used to hold conferences each Friday at the Baltimore City Hospital for interested staff from the City Hospital and Johns Hopkins Hospital. This was attended by the BCHD "lead nurse" who apprised us of the situation in the home, which was very important in deciding on long-term management. The report also mentions education. By this time, manufacturers of cribs and toys reported using paint free of lead pigment; however, it still occurs today in relation to imported toys. In 1941, Dr. Williams attempted to put through an ordinance prohibiting the use and requiring the removal of white lead paint in the interior of the buildings as had been done in some European countries earlier. Although primary prevention was intended, this was not feasible (Fee, 1990). In 1951, the Health Department adopted a regulation under the ordinance on Hygiene of Housing, which read as follows: "Interior paint. No paint shall be used for interior painting of any dwelling or dwelling unit or any part there of, unless the paint is free from any lead pigment." This actually was a considerable advance, although for reasons unknown it did not include lead dryers in paints and as noted it did not include exterior paints. This continues to be fought in the

Maryland legislature and in the courts. The Health Department also required the labeling of lead paint. Since the print on the cans was so small, the citizens were advised informally, not to use "chrome yellow," "chrome green," or "chrome orange" as those pigments contained substantial amounts of lead chromate.

Kaplan and Shaull (1961) developed a screening test for lead paint in the late 1940s. Earlier the lead chromate test had been used, but this was of no use for enforcement since it entailed a prolonged gravometric procedure. Dr. Kaplan's field test required a strip of index card, which was balanced on a piece of balsa wood (puchased at a hobby store) in such a manner that one could weigh 25.0 $\pm$ 3 mg of paint. This amount was placed in a small Pyrex glass tube, to which 3 drops of 1:1 nitric acid was added. The test also required a box of wooden matches. The field nurse then took a wooden match out, lit it, and heated the tube until the entire match was consumed, which required approximately 30 s. The tube was cooled and 1% sodium sulfite solution and 2 drops of 20% potassium iodide solution were added. A positive test was indicated by the appearance, within 1 s, of a yellow color, which indicated the presence of more than 1% of lead.

The screening samples were collected in the home and taken to the laboratory for a more thorough analysis. Since the limit was set at 1% of lead in the paints, it was not necessary for the purposes of code enforcement to use anything more than the limit test, such as this. This minitest for lead in paint provided the infrastructure for enforcing the ordinance requiring the removal of lead paint in excess of 1% of the final solids. At first enforcing this ordinance was limited to areas where the visiting nurse found tooth marks and those identified by the parent as areas where the child had been seen chewing paint. This proved to be inadequate and progressively over the years the examination was extended to the sampling of paint from all surfaces on the interior of the home.

Unfortunately the City never specified the means by which the paint should be removed. This was generally done by softening the paint with a blowtorch and scraping it off with a putty knife, which we now know filled the dwelling with particulate lead. No legally mandated cleanup was required. This meant that the only effective means of reducing the child's exposure was to move him into "lead safe" housing, whenever that could be found.

When the author began his fellowship in pediatrics for the study of lead poisoning a well-developed public health program for the time was operating in the city of Baltimore.[3] The first findings under the LIA grant were that children who ingested paint were getting more lead than even heavily exposed industrial workers. This was determined by the daily fecal content of lead. In 1955, apparently LIA did support voluntary regulations Z66.1 on limiting the use of lead pigments in interior paints to less than 1%. Needless to say, although initially suspected by LIA, we found that the BCHD was not over diagnosing lead poisoning, but that it was indeed a much more serious problem from the public health point of view as further work by the author and others demonstrated. This proved to be an enormous help. This investigator was indeed fortunate to work in Baltimore, as it had the only complete Health Department lead program in the country at the time. The author's work over the past 50 years may be considered under the following headings: (1) Exposure, (2) Renal Fanconi Syndrome, (3) Disturbances in Heme Synthesis Due to Lead, (4) Treatment, and (5) Screening.

It should be noted that Dr. Kaplan's simple paint sample test has now been replaced in the early 1970s, by the X-ray fluorescence detector. Such instruments generally cost around $1500.

The City Health Department's capacity for doing blood lead by the dithizone technique had increased to 8 samples per day, generally 4 days per week. An additional technician could be pressed into service so that in emergencies 16 samples per day could be done. It required 10 ml of blood. When we began to study the effects of chelation therapy on blood and urine lead, frequent sampling was needed. We therefore turned to the wet-digestion technique of Bessman and Layne (1955). This technique eliminated the bismuth removal step, as that was no longer necessary since bismuth was no longer being used in the treatment of syphilis. This procedure required 2 ml of blood and actually in working out the BAL-EDTA therapy we took blood every 4 h for the first 2–3 days to monitor the effects of these drugs independently and together, on blood lead concentration. This process could be carried out in a single tube; however, we did have to have a glass blower join a female fritted glass filter to the tube itself. The digestion was carried out in this tube. At

[3] When the Lead Industry Association (LIA) found it virtually impossible to believe that one city, namely Baltimore, was diagnosing over 24% of the total United States incidence of childhood lead poisoning, they had left apparently a sum of money with the Department of Pediatrics at the Johns Hopkins Hospital, to study the issue. They thought that the diagnosis was being made excessively by Dr. Huntington Williams and the Baltimore City Health Department. This funding supported the initial year of the author's fellowship.

the end the chloroform was at the bottom so that the supernatant aqueous solution could be aspirated and discarded. Deleaded cotton was then placed in the female part of the joint to filter out water. Dummy analyses were run in new tubes until they gave negative results for lead.

Exposure

The LIA left a sum of money in the Department of Pediatrics at the Johns Hopkins Hospital to study childhood lead poisoning. The grant funded the author during the first year of his studies, providing for the necessary equipment and reagents. In discussing the matter with Dr. Harold Harrison, it was decided that data were needed on intake of lead by young children. This was done by measuring fecal output. For this purpose the method of Snyder (1947) was used. We followed the precautions detailed by Clifford and Wichman (1936) which detailed the many pitfalls in blood lead analysis and means of avoiding them. The dry ashing technique was to be used for this. It required a muffle furnace, which brought up the first of my troubles. At the time the Hospital was buying electricity as alternating current (AC) and converting it to direct current (DC), as they had much old equipment. The muffle furnace has to be controlled by rheostats, because one had to avoid raising the temperature in the furnace above 480°C to prevent loss of lead due to the formation of volatile lead carbonyl compounds. Yet in order to destroy organic material it had to be as close to 480°C as possible. This process took at least 2 days. I had to stay up all night, for a number of nights, to find out when and where to reset the rheostats as the city reduced and raised the current according to variations in load throughout the 24-h period. I finally trained the night head nurses to reset the rheostat at a specified times, so the temperature would remain relatively constant.

In those days the investigator did all the analytical work including cleaning the glassware. One had to purify all the reagents, as there was no such thing as ultrapure reagents. To check on contamination a dummy analysis was run in all of the separatory funnels just before use for the actual samples. We also had to avoid acids or ammonium hydroxide in glass bottles with a B in a diamond on the bottom of the bottle. That specified glass that was likely to be leaded glass. We tried to order all the acids as well as other chemicals from G.F. Smith as their products tended to be more pure than required for ACS reagent grade at the time. Chloroform had to be ordered in bottles specifying that there be no cardboard plug under the cap as this was held on by some adhesive, which in the course of shipment would get into the chloroform and cloud it. We could obtain chloroform in bottles without cardboard plugs from Merck & Co. Solutions of other reagents had to be purified initially by extracting with diethyldithiocarbomate in chloroform or gassing the solutions with hydrogen sulfide. For the analysis of tissues I used a cigar box with solid CO_2 and on top of this a stainless-steel mortar and pestle. This, if done properly, would yield a very fine powder. The investigator visited the homes to collect the stools, which had to be homogenized in a Waring blender; however, the standard bearing contained lead and had to be replaced by a cast iron bearing. This did not last very long and had to be frequently replaced. Many of these tricks were learned from Dr. Kaplan and other people's experience in the analyses of lead. You will not find them in textbooks. With these techniques we were able to measure the total daily dietary intake of lead in controls and were able to show that the amount of lead found in the stool of small lead poisoned toddlers exceeded the amount found in the stool of heavily exposed industrial workers (Chisolm and Harrison, 1956a). We deleaded the cotton by placing it in a Buchner funnel and by washing it with dilute nitric acid. This was placed in the stem of the separatory funnel and later in the female joint as used in the Bessman and Layne technique (1955). This was to catch any water as one was extracting lead dithizonate in chloroform from the analysis tube into the optical cuvette. If any water was left, it would cloud the solution and made the analysis worthless. I used to run 24 samples in duplicates, plus 12 control tubes or 60 separatory funnels at a time.

Renal Fanconi Syndrome

In 1953 a 33-month-old child was admitted to the pediatric service at the Johns Hopkins Hospital, with recurrent acute lead encephalopathy and a blood lead concentration of 410 μg/dL of whole blood. A peculiar thing about this child was the musty smell of the child's urine, which suggested protein. Yet, all testing for protein in the urine gave a negative result. It so happened that Dr. Walter Eberlein had just returned from Fanconi's clinic in Zurich, Switzerland, and was able to carry out unidimensional paper chromatography. This child's urine was chromatographed and massive aminoaciduria was revealed. The child was treated with $CaNa_2EDTA$ followed by citrate as described elsewhere (Chisolm *et al.*, 1955). To further study

these findings it was necessary to develop two-dimensional chromatography. No cabinet suitable for this technique could be purchased. Therefore, the investigator designed a tank and all the associated parts. It was made out of stainless-steel Type L316 (resistant to phenol and mineral acids) by a mechanic whose hobby was metalworking. Further study of the child's "glycosuria" revealed that he was excreting both glucose and fructose. Tests were run to discriminate between the two, including a technique requiring the use of an ion-exchange resin, Duolite A4. This was supplied in large chunks and had to be ground down in the laboratory before putting it on the column. Aminoaciduria but not the full Fanconi triad had been previously reported (Wilson *et al.*, 1953). Likewise aminoaciduria had been reported in uranium workers (Clarkson and Kench, 1956). We initiated a study to compare the pattern found in this lead-poisoned child with a number of others. Lead produced the pattern of renal aminoaciduria due to injury to the proximal renal tubule (Chisolm and Leahy, 1962). In the 23 patient studied none showed the full Fanconi triad of acute lead poisoning until blood lead concentrations exceeded 150 μg Pb/dL. In summary the features of these cases of the renal Fanconi syndrome resulting from lead poisoning were aminoaciduria, mellituria, hypophosphatemia in the presence of hyperphosphaturia, and the skeletal changes of acute rickets.

Disturbances in Heme Synthesis Due to Lead

It is clear that if clinical diagnosis was to advance beyond the stage of acute clinical symptoms—which in children meant symptoms of encephalopathy—preclinical biochemical indicators of lead toxicity would be needed. It was clear, for example, that early diagnosis was unlikely be made on the basis of blood lead in the early 1950s, because of limitations in the number of tests that could be done per day. One technician could do 8 tests per day and in an emergency, may be 16. Garrod (1892) was the first to demonstrate that porphyrinuria occurred in human cases of lead poisoning. Stokvis (1895) demonstrated porphyrinuria in both clinical and experimental plumbism. In the early 1950s there were important indications that lead might cause multiple partial interference in heme synthesis. In 1951 Schwartz, Zieve, and Watson published a quantitative method for the determination of coproporphyrin in urine, which I began using almost immediately (Chisolm and Harrison, 1956b). There had been considerable difficulty in finding red-sensitive photomultiplier tubes in fluorometers, which would be suitable for the measurement of porphyrin fluorescence, the usual method by which it was being measured. The author was fortunate in obtaining with Dr. Schwartz's assistance a Calectron photofluorometer, which had been developed by the Minnesota group, specifically for porphyrin analysis and manufactured by a mechanic in a garage near the university. Dr. Schwartz told me some years later that he did not think there were ever more than five of these instruments made. He also supplied me with a very small amount of highly purified coproporphyrin III to use as a primary standard. That supply would not last very long; however, we did find an ancient bottle of eosin on the shelf, which may well have dated from before World War I. In any event, it was stable and had fluorescence spectra somewhat similar to those of the porphyrins. This we used until patient with chronic lead poisoning came along who put out an unusually large quantity of coproporphyrin. Paper chromatography demonstrated that he excreted coproporphyrin III almost exclusively. Therefore when he was admitted to the hospital, I extracted his urine according to the procedure of Schwartz, Zieve, and Watson (1951) and measured its content spectrophotometrically, using the absorption coefficient (Jope and O'Brien, 1945) to determine the concentration as had been customary for many years. With this technique Chisolm and Harrison demonstrated that the quantitative 24-h urinary output of coproporphyrin was a better predictor of the response to $CaNa_2EDTA$ than the blood lead concentration. In 1956, Mauzerall and Granick published an ion-exchange resin chromatographic technique for measuring δ-aminolevulinic acid (ALA) in urine. Later on this was modified by inserting a resin to remove neutral aminoketones, which were related largely to diet and not to lead toxicity (Urata and Granick, 1963).

Treatment

For studies on chelation therapy we turned to the technique of Bessman and Layne (1955) as it required only 2 ml of blood, which was feasible in small children. One pitfall not covered was the later introduction of EDTA in the dye by companies, making pH indicators. This bound the lead and essentially destroyed the dithizone procedure as the EDTA had greater affinity than dithizone for lead. To avoid this problem it was necessary to raise the boiling point of the acid digestion by adding perchloric acid to the standard nitric-sulfuric acid digestion procedure. With these techniques, first EDTA therapy was evaluated (Chisolm and Harrison, 1956b) and later

the combined BAL-EDTA therapy (Chisolm, 1968a), which probably reduced mortality from acute lead encephalopathy in children from perhaps 30% to less than 1%. With this technique, whether a child lived or died probably depended upon how rapidly the diagnosis of encephalopathy was made.

The development and success of the combined $CaNa_2$ EDTA-BAL therapy are based on three major considerations. (1) Lead is one of two metals, the other being tin in the divalent state, which will bind with equal facility to $CaNa_2$ EDTA and BAL. (2) These drugs do not have overlapping toxicities. (3) Because of these considerations one can double the chelant to metal ratio, thereby substantially increasing excretion of lead and so improve the effectiveness of the treatment.

In 1958, when we began to work on the combined $CaNa_2$EDTA-BAL technique, a biochemical indicator was needed to monitor changes in toxicity in relation to this new therapy. It was shown that urinary ALA was much more specific for lead than urinary coproporphyrin, which is responsive to a wide number of illnesses. Initially it could not be decided whether urinary coproporphyrin (UCP) or urinary ALA would be the quicker responder to doses of BAL or $CaNa_2$EDTA. Therefore the author developed a siphon so that urine for UCP and ALAU could be collected simultaneously. This split the stream so that it would be passed into two containers: one preserved with dilute hydrochloric acid, for preservation of ALA, and the other preserved with sodium bicarbonate for the preservation of UCP. This never worked because staff and visitors usually kicked over the apparatus, which was under the bed. In any event, we learned enough to know that a urinary indicator would not respond rapidly enough. Therefore the technique of Mauzurall and Granick was adapted for the measurement of ALA in plasma (Chisolm, 1968b). This was a laborious technique but nevertheless essential to the evaluation of the combined BAL-$CaNa_2$EDTA chelation technique. With this we were able to show that following injections of $CaNa_2$EDTA, plasma ALA tended to rise whereas injection of BAL, 4-h later, made it decrease. When the two were given simultaneously plasma ALA decreased. Semiquantitative, two-dimensional paper chromatography was used to monitor renal effects. These studies showed that the combination progressively improved the biochemical indicators of renal injury. These data were first presented before the American Pediatric Society in Atlantic City in 1963 (Chisolm, 1963). Because of this greater specificity toward lead intoxication, quantitative 24-h urinary ALA was used in the author's studies up until about 1990.

In 1948 de Langen and ten Berg published a simple screening technique for measurement of coproporphyrin in the urine of workers, in which 24-h urine samples were collected and usually examined fresh, if necessary a few crystals of thymol were added, and the urine was stored in the dark. Upon analysis 10 ml of the specimen was acidified with 5 ml of glacial acetic acid and extracted into 10 ml of ether. The porphyrin was next extracted from ether by four shakings with 5% hydrochloric acid. This was not a pure extract according to them; nevertheless, a red fluorescence could be detected with the Woods lamp. McCord (1951) further modified the technique by the addition of hydrogen peroxide. The paper does not state the quantity used. The McCord modification was used by Bradley *et al.* (1956) in a study of 333 children in Baltimore, in which they evaluated the presence of coproporphyrinuria as a screening test. They were disappointed and noted that the history of pica seemed to correlate better with the blood lead concentration than UCP in freshly voided specimens of urine. They did not use 24-h collections. They concluded that asking a question about pica might provide better results. They also noted that 8 of the 604 children examined came back in with symptoms of lead encephalopathy within a year, thereby indicating the need for repeated testing. It is of further interest to know that in this inner city population, in 1950s, the average blood lead concentration was 43 μg/dL. They did however make a note that these were all inner city children and that a modest number of samples from children, not highly exposed to lead, yielded an average blood lead concentration of approximately 30 μg/dL. With these data in mind, we developed a screening test, based upon the quantitative procedure of Schwartz, Zieve, and Watson (1951).

Screening

The following technique was used from the mid 1950s to the late 1960s in the pediatric clinics with which the author was associated. This technique took into account the findings of Watson *et al.* (1951), who found that under normal circumstances about half of the coproporphyrin excreted in urine was in the unoxidized nonfluorescent state. It was necessary to oxidize this. They tried peroxide and reported inconsistent results; instead they developed a technique requiring a specific amount of 0.1% iodine in 95% ethanol and the use of peroxide-free ether. Very

shortly after developing and reporting this test, we changed to ethyl acetate, which gave equivalent results. With this technique qualitative test results of 0,1,2,3, and 4+ porphyrin fluorescence intensity could be estimated with the Woods lamp (Benson and Chisolm, 1960). A 3 and 4+ fluorescence indicated a blood lead concentration above 80 μg/dL, and a 2+ test was indiscriminate, but was positive up to a blood lead concentration of about 80 μg/dL. This test was useful if carried out immediately. The author prepared sealed ampoules of pure coproporphyrin III at the various concentrations for use as standards by house officers in the emergency rooms at the pediatric clinics. The author also trained all the pediatric assistant residents in these two hospitals in the use of the technique. The rule then was, if an inner city child came in during summertime with vomiting, he/she had to have a qualitative urinary coproporphyrin test, even if it necessitated aspiration of the bladder or catheterization. After the time the test was developed, standards of coproporphyrin could be obtained commercially, although we did find that mesoporphyrin IX could be used instead, provided that the sensitivity was adjusted appropriately. Mesoporphyrin IX was then available commercially from an English company, which went out of business sometime in the 1960s.

At the end of the 1960s UCP was supplanted by a qualitative urinary ALA screening test. The kit for this was available commercially; however, we were able to show in a study published subsequently (Chisolm *et al.*, 1976) that the concentration of ALA in each individual voiding during the same 24-h period showed wide variation while the total 24-h output showed highly statistically significant relationships with both blood lead and urinary lead output under the influence of chelating agents. In fact 24-h urinary ALA was actually the best predictor available of the amount of lead excreted under the influence of EDTA.

In 1959 Whitaker and Vietti published a technique based on the use of the fluorescence microscope for the measurement of the fluorescence in erythrocytes as a rapid screening test for lead poisoning in children. We tried this, but were never successful. Various investigators that I talked to also had no success with this technique, which finally was replaced with screening adaptations of erythrocyte protoporphyrin, first published in 1973 by Sassa *et al.* Chisolm and Brown (1975) in *Selective Methods in Clinical Chemistry* published the technique, which has been used in this laboratory since 1975.

ATOMIC ABSORPTION AND ELECTROCHEMICAL ERA

In January 1971 President Nixon signed into law the Lead-Based Paint Poisoning Prevention Act of 1970. This program was for the most part first awarded to the Bureau of Community and Environmental Management (BCEM) in Cincinnati, a bureau that was eliminated in about 1973 in a government reorganization. In 1973 most of the program, except for the research arm, was awarded to the Centers of Disease Control and Prevention in Atlanta where it has been lodged ever since. In 1970 Keppler *et al.* published a comparison between the dithizone and the emerging atomic absorption spectrophotometric (AAS) methods for measuring lead in blood, which revealed the sad state of affairs in blood lead analysis. In the author's laboratory primary standards consisted of blood from occupationally exposed human lead workers, analyzed by the reference technique of thermal ionization mass spectroscopy (TIMS). These were done by Dr. William I. Manton for us in the 1970s and 1980s. By this time our source of human blood no longer existed. Fortunately the National Bureau of Standards and then the National Institute of Standards and Technology (NIST) made available samples analyzed by TIMS as primary reference standards. Also the Keppler report (1970) recommended that proficiency testing be carried out. This was organized and first monitored by the CDC in 1975. All programs wishing to receive grants from the CDC had to participate in these programs. This was formalized in the Clinical Laboratory Improvement Act (CLIA) in 1988. Satisfactory participation in blind interlaboratory proficiency testing programs has been required for licensing ever since. The author's laboratory has participated as a reference laboratory for blood lead, for the State of New York and the Wisconsin-MCH (predecessor Wisconsin-CDC) programs for the past 20–25 years. A study by Parsons (1992) revealed that over the 15–20 years or so of the New York State program, the number of laboratories providing proficient results increased from 80% to about 95%.

When the lead poisoning prevention act was signed into law, in 1971, among other things it mandated universal screening of children by blood lead measurement. It had become evident during the early 1970s, as mentioned before, that urinary tests while satisfactory to monitor adult workers, would be totally inadequate for the screening of children, particularly those less than 3 years of age. One of the first things, Dr. Barry Wood, who was in charge of

the program at BCEM did, was to explore the question of micro blood lead techniques. It turned out that two young graduate students at the University of Michigan were using an electrochemical technique, namely anodic stripping voltametry (ASV), to estimate the concentrations of trace metals in lake water. He believed that this could be adapted to the measurement of lead in blood, which was eventually achieved, with a large bit of help from the author's laboratory particularly in the region of digestion of blood, with which these young men had no experience. While this technique was on the drawing board, the Delves cup adaptation of flame atomic absorption spectrophotometry (FAAS) had been reported from England in 1970 (Delves, 1970). The initial instrumentation was attached to commercial AAS instruments by flimsy mechanisms. At the Baltimore City Health Department Dr. Kaplan had a jeweler's mount made to order to provide better positions of the Delves cup in the flame. It may be said that micro blood lead determinations were finally perfected with the development of graphite furnace atomic absorption spectrophotometry (GFAAS) with the L'wow platform in miniprocessor-controlled instruments, which have been available for the past 10 years or so. The lead determinations on biological samples in careful laboratories have been highly reproducible, precise, and accurate since these developments. Indeed the author is aware of one laboratory that with the latest GFAAS equipment can produce blood lead measurements with a standard deviation of $\pm$ 0.6 μg/dL. The author's instrument is not quite so modern and produces blood lead measurements with a standard deviation $\pm$ 0.9 μg/dL.

The author received a grant from BCEM, to assist in the development of anodic stripping voltametry. The first instrument that Environmental Science Associates (ESA) provided required complete digestions of the sample and a 30- to 40-min plating time before the polarity of the current was reversed to measure lead, based on the current generated with this reversal. ESA set up a service laboratory, using banks of 32 electrodes operated in three shifts around the clock. This clearly would not answer the question of universal screening. They produced a new one, Model 3010, which consisted of a single electrode, did not require digestion of the sample, and gave a result in 90-s. Initially ESA cleaned up all the reagents and supplied them and cleaned sampling kits for these measurements. Thus the user did not have to buy anything but the complete kit and instrument from the manufacturer.

The author found that the grant under which his lead clinic was operating in the 1970s could not possibly tolerate the expense of purchasing the supporting electrolyte solution, which was necessary for operation of this instrument. This electrolyte solution in the samples with tripotassium ethylenediaminetetraacetic acid (K_3EDTA) as anticoagulant could not be used as received. It was determined in this laboratory that one had to compensate for EDTA. K_3EDTA also preserved samples for FEP measurements. Samples so collected would be good for at least 4–6 weeks and quite possibly longer when kept in deep freeze. The author therefore set forth to make his own reagent. We first tried to do this by purchasing ultrapure reagents. These reagents could be purchased from the 1970s onward, whereas 25 years earlier lead laboratories had to purify all their own reagents. Commercially ultrapure reagents were available. In the author's experiences unfortunately some "ultrapure" reagents varied in their purity from one lot to another. So it was still necessary to purify our own reagents. For this purpose we turned to reagent based upon an HCl-KCl (hydrochloric acid-potassium chloride) pH 1.3 buffer, with a trace of nickel chloride. Ultrapure hydrochloric acid could of course be purchased. We used KCl that was prepared by passing through Chelex 100, an ion-exchange resin, which would remove all divalent and trivalent cations but not monovalent cations, such as potassium. This reagent continues to be made in this laboratory. The cost is about 8 cents per sample and the results obtained are highly satisfactory (Bannon *et al.*, 1994). The National Health and Nutrition Examination Survey (NHANES) of 1980 (NHANES II) and 1991 (NHANES III) has revealed substantial reductions in blood lead concentrations, although universal screening has not been employed. Nevertheless the current CDC approach is to promote screening targeted to high-risk areas. In some states this is now required by law, but perhaps not well enforced.

In the original Lead Paint Poisoning Prevention Act in 1970 one of the titles awarded all research to the Department of Housing and Urban Development (HUD). Over many years HUD used these funds to assist the Environmental Protection Agency in showing the relationship among airborne lead, automotive exhaust, and blood lead concentrations in adults as the primary cause of lead toxicity in human beings. It is only within the past decade that HUD has set up an office of prevention of lead paint poisoning in children, this under congressional mandate. They are to receive and accept advice from EPA and CDC in these projects. Significant steps have

been made in reducing the hazard of lead exposure in housing during this time (Farfel and Chisolm, 1991).

In summary the atomic absorption spectrophotometric and the electrochemical era have seen substantial advancements in lead analysis, which have provided the basis for improvements in research in the lead toxicity and public health steps to reduce its occurrence.

SUMMARY

We have seen that in the pre-dithizone era, the diagnosis in children depended almost entirely on the recognition of symptomatic cases. With the advent of dithizone in the 1930s encephalopatic, mildly symptomatic, and asymptomatic cases could be recognized and documented with blood lead values. During the same years it became evident that basophilic stippling of the circulating erythrocytes and Burton's line on the gum were virtually useless in the diagnosis of lead poisoning in children. Also increased density at the ends of the growing long bones was identified as the feature of excess lead storage. The dithizone procedure was too time consuming for screening large numbers of children. During this era the urinary UCP test and ALAU tests was used for screening symptomatic children in hospitals. It was of great benefit in diagnosis and management of suspected cases of lead poisoning. It was during this era that the chelating agents $CaNa_2EDTA$ and BAL were developed. This permitted the public health approach to the management of childhood lead poisoning as previously described (Williams *et al.*, 1952), which included among other things the measurement of lead in paint samples for identification of lead hazards in housing. This too was greatly improved later on, with the development of the X-ray fluorescence lead analyzer, with which a large number of samples could be processed in a few hours. That was not the case with previous techniques.

Today the laboratory capacity for screening with micro blood lead measurements is more than adequate. On the other hand, public health procedures have generally not been able to develop programs in which more than one-half to two-thirds of the children at high risk are reached (US GAO Report, 1999). In particular in case management exerting parents to prevent their children from eating lead in the home has proven to be a complete failure as a means of reducing blood lead concentrations (Schucker *et al.*, 1965; Rhoads *et al.*, 1999).

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