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Kinetics of Aluminum-Induced Inhibition of δ-Aminolevulinic Acid Dehydratase *In Vitro*

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ABSTRACT. Anemia, one consequence of aluminum toxicity, may be due to inhibition of enzymes in the heme biosynthetic pathway. In this study, the *in vitro* effect of aluminum on rat liver and erythrocyte δ-aminolevulinic acid dehydratase (δ-ALA dehydratase), an enzyme that is sensitive to a number of metal ions, was investigated. The presence of 1–10 μM $AlCl_3$ caused a concentration-dependent inhibition of liver δ-ALA dehydratase activity. The K_i for $AlCl_3$-induced inhibition of δ-ALA dehydratase was 4.1 μM, and 10 μM $AlCl_3$ virtually abolished δ-ALA dehydratase activity (99% inhibition). Erythrocyte δ-ALA dehydratase was also inhibited by similar concentrations of $AlCl_3$ and displayed a K_i of 1.1 μM. $AlCl_3$ (5 μM) decreased the V_{max} by 50% but did not change the K_m, suggestive of reversible, noncompetitive inhibition. Sodium citrate (50 μM) when added with $AlCl_3$ completely restored δ-ALA dehydratase activity to basal levels. Thus, disruption of δ-ALA dehydratase occurred at low micromolar levels of $AlCl_3$ *in vitro*, which may help to explain abnormalities in the heme pathway in cases of aluminum poisoning. BIOCHEM PHARMACOL 52;6:927–931, 1996.

KEY WORDS. δ-aminolevulinic acid dehydratase; porphobilinogen synthetase; aluminum inhibition; heme biosynthesis; porphyrins

δ-ALA dehydratase[2] (EC 4.2.1.24), also known as porphobilinogen synthase, catalyzes the formation of porphobilinogen from two molecules of δ-aminolevulinic acid. It is a metalloenzyme, requiring zinc ions for activity [1–3] and is highly sensitive to a variety of other metal ions. In addition to Zn^{2+}, Fe^{2+} can also activate δ-ALA dehydratase [4], while other divalent cations such as Cu^{2+} and Pb^{2+} inhibit the enzyme [5, 6]. Pb^{2+} is a particularly potent inhibitor of δ-ALA dehydratase with an IC_{50} of 1.9 μM *in vitro* [7]. Therefore, inhibition of human erythrocyte δ-ALA dehydratase is used as a biomarker of lead poisoning *in vivo* [8]. Cd^{2+} activates δ-ALA dehydratase at concentrations of less than 40 μM but inhibits the enzyme at higher concentrations *in vitro* [7].

Trivalent cations such as In^{3+} also inhibit δ-ALA dehydratase *in vitro* [9]. However, conflicting results have been reported with Al^{3+}. The *in vitro* studies of Davis and Avram [7] indicate that even millimolar concentrations of aluminum exert no effect on the enzyme, whereas Abdulla *et al.* [10] note that this metal ion causes inhibition of δ-ALA dehydratase. Meredith *et al.* [11] show a stimulatory effect of aluminum of δ-ALA dehydratase *in vitro*. Conflicting results have also been reported for the *in vivo* effects of aluminum on δ-ALA dehydratase [12, 13].

Aluminum has been shown to induce anemia and encephalopathy in humans and animals [14, 15]. The anemia associated with aluminum toxicity may be due to aluminum-induced disruption of the heme biosynthetic pathway [16]. Most cases of human disease associated with aluminum toxicity have been seen in hemodialysis patients and industrial workers [15]. However, because the normal and toxic levels of brain aluminum differ by only 3- to 10-fold, it is possible that chronic exposure to low levels of aluminum may exacerbate some late-onset neurological disorders [15].

The present study was undertaken in an effort to resolve the conflicting reports regarding the *in vitro* effects of aluminum on δ-ALA dehydratase. The kinetic parameters of aluminum-induced effects on this enzyme can contribute to our understanding of the interaction of aluminum with δ-ALA dehydratase.

MATERIALS AND METHODS

Materials

ALA, *N*-ethylmaleimide, trichloroacetic acid, heparin and *p*-dimethylaminobenzaldehyde were obtained from the Sigma Chemical Co., St. Louis, MO. Aluminum chloride (99.99%) and perchloric acid (70%) were purchased from the Aldrich Chemical Co., Milwaukee, WI.

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[2] *Abbreviations:* δ-ALA dehydratase, δ-aminolevulinic acid dehydrase; and ALA, δ-aminolevulinate hydrochloride.

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Assay Methods

Male, Sprague-Dawley rats (200–250 g), obtained from Harlan, Inc., Indianapolis, IN, were housed under a 12-hr light/dark cycle and were given standard rat chow *ad lib.* Rats were anesthetized with intraperitoneal injections of Nembutal (sodium pentobarbital), 4 mg/100 g body weight, prior to being killed. Whole blood, collected in tubes containing 1 mg/mL heparin, and livers were quick-frozen on dry ice and stored in polystyrene tubes until used.

Aliquots (6 g) of frozen liver were suspended in 2 mL of 0.15 M NaCl and homogenized with 10 up–down strokes of a hand-held tissue grinder. Protein concentrations were determined according to the method of Lowry *et al.* [17] with minor modifications. Liver homogenates and whole blood were diluted in 0.15 M NaCl, and sodium deoxycholate (2% final concentration) was added to all samples. Bovine serum albumin served as standard.

δ-ALA dehydratase was assayed as described by Labbe [18] but 0.25 M HEPES buffer, pH 7.0, replaced the citrate-phosphate buffer used in the original method. Deionized water was used to make all solutions, and the assays were performed in polystyrene tubes. $AlCl_3$ solutions were prepared immediately before use. In addition to 8–9 mg total liver or blood protein, the assay mixture contained 0.11% Triton X-100, $AlCl_3$ or an equivalent volume of deionized water, and 0.1–6.5 mg ALA in a total volume of 2.5 mL. Immediately, 1 mL of this assay mixture was removed and added to 1 mL of 0.25% *N*-ethylmaleimide/10% trichloroacetic acid, which served as the assay blank. The remaining assay mixture was incubated at 37° for 75 min, and then 1.5 mL of the *N*-ethylmaleimide/trichloroacetic acid solution was added. Tubes were spun in a table-top centrifuge for 5 min to precipitate tissue. An aliquot of the supernatant was added to an equal volume of modified Ehrlich's reagent [19]. After 13 min, the absorbance at 555 nm was determined. The molar extinction coefficient of the colored product was 61,000 [19]. Under these conditions, δ-ALA dehydratase activity was linear with reaction times for 30–120 min.

Results are expressed as the means ± SEM of experiments performed at least three times with similar results. Statistical significance was assessed using Student's *t* test.

RESULTS

The assay for δ-ALA dehydratase, as described by Labbe [18], used 0.25 M citrate-phosphate, pH 6.65. However, because aluminum formed insoluble complexes with phosphate, another buffer was needed which did not precipitate aluminum. Several buffers were tested, including imidazole-HCl, imidazole-citrate, and HEPES. The activity of δ-ALA dehydratase in 0.25 M HEPES at pH 6.8 to 7.2 was comparable to that in the citrate-phosphate buffer. Thus, 0.25 M HEPES, pH 7.0, was used in all assays.

The presence of $AlCl_3$ caused a concentration-dependent inhibition of liver δ-ALA dehydratase activity (Fig. 1). Significant inhibition, 20.7% ($P < 0.05$), of enzyme activity occurred at 1 μM $AlCl_3$, 48.4% ($P < 0.001$) inhibition was noted at 5 μM $AlCl_3$, and 10 μM $AlCl_3$ virtually abolished (99% inhibition) δ-ALA dehydratase activity.

A double-reciprocal plot of liver δ-ALA dehydratase activity versus substrate concentration in the presence or absence of 5 μM $AlCl_3$ indicated a 50% decrease in the V_{max} in the presence of $AlCl_3$, but no change in the K_m (Fig. 2). This suggested that $AlCl_3$ was acting as either a reversible, noncompetitive or an irreversible inhibitor of δ-ALA dehydratase.

To distinguish between these two possible modes of inhibition, a plot of V_{max} versus total protein concentration was constructed (Fig. 3). This plot intersected the origin, which is suggestive of reversible, noncompetitive inhibition [20]. To confirm this finding, 50 μM sodium citrate was preincubated with protein and 5 μM $AlCl_3$ for 1 min prior to the addition of substrate (Table 1). The presence of sodium citrate did not alter basal levels of δ-ALA dehydratase activity. The presence of 5 μM $AlCl_3$ alone reduced δ-ALA dehydratase activity by 50%. The addition of sodium citrate with $AlCl_3$ completely restored δ-ALA dehydratase activity to basal levels.

A Dixon plot gave a K_i of 4.1 μM (Fig. 4), which is in good agreement with the observation that 5 μM $AlCl_3$ inhibited 50% of liver δ-ALA dehydratase activity (Table 1).

δ-ALA dehydratase from rat erythrocytes also was inhibited by low micromolar concentrations of $AlCl_3$ *in vitro* (Fig. 5). A Dixon plot indicated that the K_i for aluminum-induced inhibition of the erythrocyte enzyme was 1.1 μM (Fig. 5, inset).

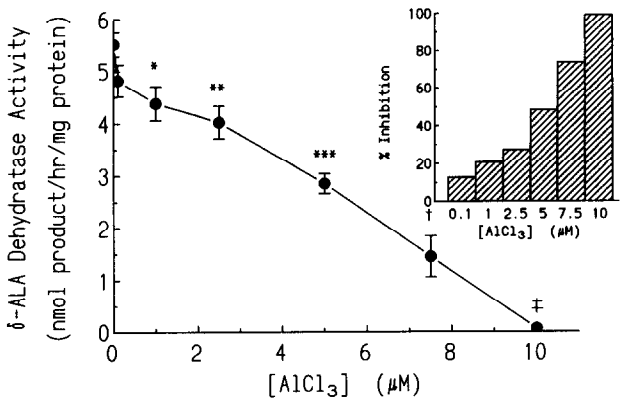

FIG. 1. Rat liver δ-ALA dehydratase activity as a function of $AlCl_3$ concentration. $AlCl_3$ and 1.7 mg ALA were added at the time of assay. Basal levels of δ-ALA dehydratase activity were determined by substituting an equal volume of distilled water for the $AlCl_3$. Data are the means ± SEM from 3 separate experiments. Key: significant change from basal: (*) $P < 0.05$; (**) $P < 0.02$; (***) $P < 0.001$; (†) $P < 0.002$; and (‡) $P < 0.0001$. Inset: Data plotted as percent inhibition of δ-ALA dehydratase activity versus $AlCl_3$ concentration.

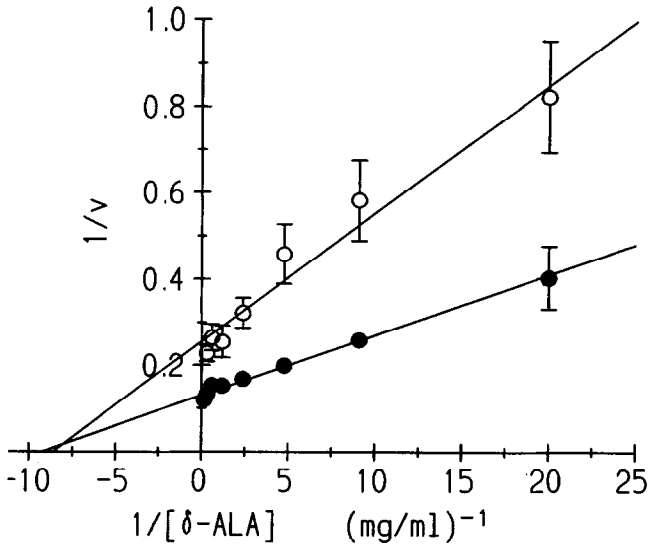

FIG. 2. Double-reciprocal plot of δ-ALA dehydratase activity in the presence (open circles) or absence (closed circles) of 5 μM $AlCl_3$. Data are the means ± SEM of 5 separate experiments.

DISCUSSION

The activity of rat liver and blood δ-ALA dehydratase was significantly inhibited *in vitro* by $AlCl_3$ at concentrations greater than or equal to 1 μM. The K_i values of aluminum-induced inhibition of liver and erythrocyte δ-ALA dehydratase were 4.1 and 1.1 μM, respectively. The reason for the difference between these K_i values is uncertain. It is possible that different δ-ALA dehydratase isoenzymes are expressed in different tissues or that the milieu of the enzyme is altered in the liver and erythrocyte. Although the presence of rat δ-ALA dehydratase isoforms has not been determined, isoforms of this enzyme do exist in humans, and Astin *et al.* [21] hypothesize that they differ in their sensitivity to lead. Furthermore, rat erythrocyte and liver δ-ALA dehydratase differ in their response to dithiothreitol, which stimulates erythrocyte δ-ALA dehydratase but not the liver enzyme [22].

TABLE 1. Reversal of $AlCl_3$ inhibition of δ-ALA dehydratase by sodium citrate

$AlCl_3$ (5 μM)	Sodium citrate (50 μM)	δ-ALA dehydratase activity (nmol product/hr/mg protein)
–	–	5.77 ± 0.38
–	+	6.12 ± 0.07
+	–	2.14 ± 0.23*
+	+	5.37 ± 0.46

Liver homogenate containing $AlCl_3$ or an equal volume of water in the presence or absence of sodium citrate was preincubated for 1 min at 37° prior to the addition of 1.7 mg ALA. Data are the means ± SEM from 3 separate experiments.
* Significant change from basal activity, $P < 0.002$.

The K_i values for aluminum-induced inhibition of δ-ALA dehydratase are comparable to the K_i of 1.9 μM for lead-induced inhibition of the enzyme [7]. However, the actual concentration of Al^{3+} in the assays is considerably less than the concentration of $AlCl_3$ because of the formation of a number of aluminum complexes in neutral solution [23]. The maximum concentration of free Al^{3+} in a 1 mM solution at pH 7.0 is estimated to be approximately 10^{-12} M [24]. Thus, aluminum is a very potent inhibitor of δ-ALA dehydratase.

Our data are in agreement with the results of Abdulla *et al.* [10], who noted significant inhibition of the human erythrocyte enzyme following a 4-hr preincubation of whole blood with 4 mM $AlCl_3$ prior to assay. The much higher concentrations of $AlCl_3$ needed to produce significant in-

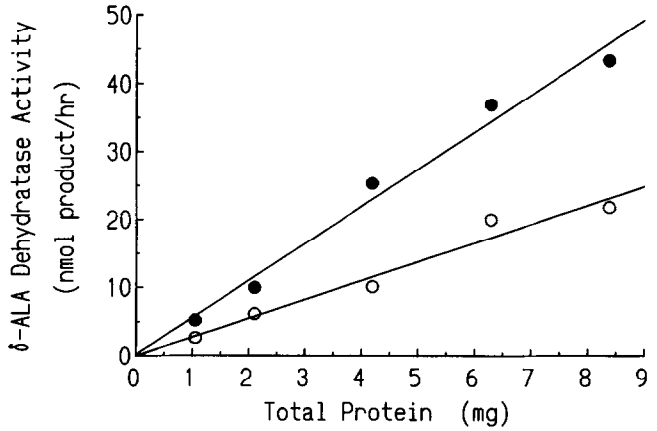

FIG. 3. δ-ALA dehydratase activity as a function of total protein concentration. Various concentrations of total protein and 1.7 mg ALA were assayed in the presence (open circles) and absence (closed circles) of 5 μM $AlCl_3$. Data are from a representative experiment repeated 3 times with similar results.

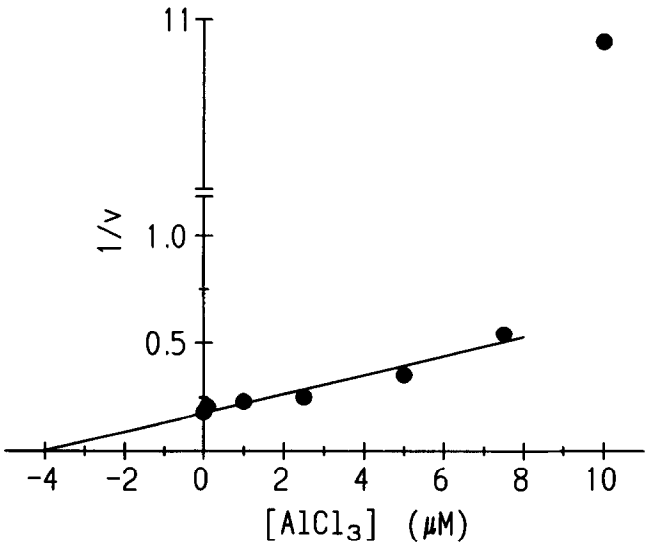

FIG. 4. Dixon plot of aluminum-induced inhibition of liver δ-ALA dehydratase. In the case of noncompetitive inhibition, the x-intercept is equal to the K_i [20]. The data point at 10 μM $AlCl_3$ represents over 98% inhibition of δ-ALA dehydratase and is not included in the linear regression analysis.

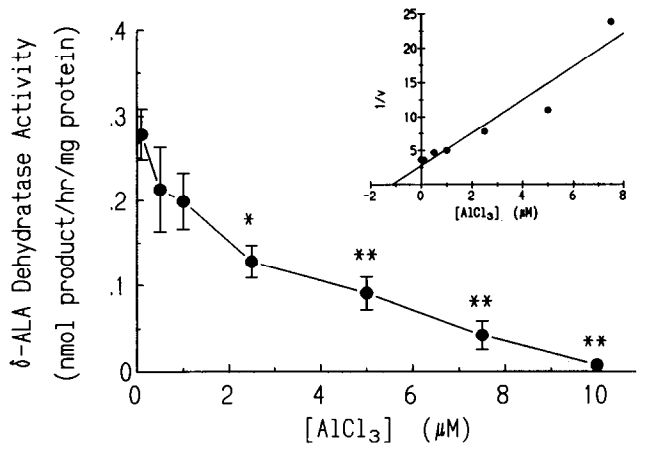

FIG. 5. Rat erythrocyte δ-ALA dehydratase activity as a function of $AlCl_3$ concentration. $AlCl_3$ and 1.7 mg ALA were added at the time of assay. Basal levels of δ-ALA dehydratase activity were determined by substituting an equal volume of distilled water for the $AlCl_3$. Data are the means ± SEM from 7 separate experiments. Key: Significant change from basal: (*) $P < 0.003$; and (**) $P < 0.0001$. Inset: Dixon plot of $AlCl_3$-induced inhibition of rat erythrocyte δ-ALA dehydratase activity.

hibition of δ-ALA dehydratase may be due to the use of whole blood in that earlier work. Aluminum would have to cross the erythrocyte membrane, so that the effective concentration of aluminum available to interact with δ-ALA dehydratase would be diminished markedly. In this present work, $AlCl_3$ was added to hemolyzed whole blood at the time of assay.

Our results are in contrast to the work of Davis and Avram [7], who reported no effect of $AlCl_3$ on δ-ALA dehydratase activity and also that of Meredith *et al.* [11] who saw a stimulation of erythrocyte δ-ALA dehydratase by $AlCl_3$. In both of these studies, the δ-ALA dehydratase assay was performed using phosphate buffer. Aluminum ions form insoluble complexes with phosphate [15]. Consequently, depending on the concentrations of $AlCl_3$ and phosphate used, considerable amounts of aluminum would be complexed and would be unavailable to affect the enzyme. This may explain the results of Davis and Avram [7]. The high concentrations of $AlCl_3$ needed by Meredith *et al.* [11] to provoke stimulation of δ-ALA dehydratase would be in keeping with this notion.

This current study indicates that $AlCl_3$ is acting as a reversible, noncompetitive inhibitor of δ-ALA dehydratase. This implies that aluminum is not binding to the active site of the enzyme. Others have shown that δ-ALA dehydratase is a metalloenzyme that binds 8 zinc ions [25, 26]. The work of Jaffe *et al.* [25, 26] suggests that half of these ions prevent the oxidation of sulfhydryl groups that are essential for enzyme activity and that the other zinc ions bridge the 8 subunits of the enzyme to maintain its quaternary structure. By competing with any or all of these zinc ions, aluminum ions could inhibit the activity of δ-ALA dehydratase. In support of this idea, the *in vitro* inhibitory effect of Ga^{3+} and In^{3+} could be reversed by the addition of Zn^{2+} [7]. Additional studies are needed to determine if Zn^{2+} can overcome the inhibitory effects of $AlCl_3$ on δ-ALA dehydratase and to determine the functional groups on the enzyme to which aluminum binds.

Although Al^{3+} is very insoluble in neutral, aqueous solutions such as serum [23] and the presence of various ions in serum presumably could compete with δ-ALA dehydratase for Al^{3+}, alterations in δ-ALA dehydratase activity are seen in animals exposed to aluminum salts *in vivo* [13]. Furthermore, in patients on chronic hemodialysis, a significant correlation is noted between serum aluminum concentrations and decreases in erythrocyte δ-ALA dehydratase activity [16]. Thus, exposure to aluminum *in vivo* also affects δ-ALA dehydratase. Additional studies are needed to probe the mechanism and reversibility of aluminum-induced inhibition of δ-ALA dehydratase *in vivo*.

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References

1. Anderson PM and Desnick RJ, Purification and properties of δ-aminolevulinic acid dehydratase from human erythrocytes. *J Biol Chem* **254:** 6924–6930, 1979.
2. Tsukamoto I, Yoshinaga T and Sano S, The role of zinc with special reference to the essential thiol groups in δ-aminolevulinic acid dehydratase of bovine liver. *Biochim Biophys Acta* **570:** 167–178, 1979.
3. Bevan DR, Bodlaender P and Shemin D, Mechanism of porphobilinogen synthase. *J Biol Chem* **255:**2030–2035, 1980.
4. Calissano P, Cartasegna C and Bonsignore D, Azione di alcuni metalli sull'ALA-deidratase eritrocitaria purificata da sangue umano. *Lav Um* **17:** 493–497, 1965.
5. Gibson KD, Neuberger A and Scott JJ, The purification and properties of δ-aminolevulinic acid dehydratase. *Biochem J* **61:** 618–629, 1955.
6. DeBruin A, Effect of lead exposure on the level of δ-aminolevulinic dehydratase activity. *Med Lav* **59:** 411–418, 1968.
7. Davis JR and Avram MJ, Correlation of the physicochemical properties of metal ions with their activation and inhibition of human erythrocytic δ-aminolevulinic acid dehydratase (ALAD) *in vitro*. *Toxicol Appl Pharmacol* **55:** 281–290, 1980.
8. Nakao K, Wada O and Yano Y, δ-Aminolevulinic acid dehydratase activity in erythrocytes for the evaluation of lead poisoning. *Clin Chim Acta* **19:** 319–325, 1968.
9. Connor EA, Yamauchi H and Fowler BA, Alterations on the heme biosynthetic pathway from the III-V semiconductor metal, indium arsenide (InAs). *Chem Biol Interact* **96:** 275–285, 1995.
10. Abdulla M, Svensson S and Haeger-Aronsen B, Antagonistic effects of zinc and aluminum on lead inhibition of δ-aminolevulinic acid dehydratase. *Arch Environ Health* **34:** 464–469, 1979.
11. Meredith PA, Moore MR and Goldberg A, Effects of aluminum, lead and zinc on δ-aminolaevulinic acid dehydratase. *Enzyme* **22:** 22–27, 1977.
12. Flora SJS, Dhawan M and Tandon SK, Effects of combined exposure to aluminum and ethanol on aluminum body burden

and some neuronal, hepatic and haematopoietic biochemical variables in the rat. *Hum Exp Toxicol* **10:** 45–48, 1991.
13. Zaman K, Zaman W, Dabrowski Z and Miszta H, Inhibition of delta aminolevulinic acid dehydratase activity by aluminum. *Comp Biochem Physiol* **104C:** 269–273, 1993.
14. Kaiser L and Schwartz KA, Aluminum-induced anemia. *Am J Kidney Dis* **6:**348–352, 1985.
15. Ganrot PO, Metabolism and possible health effects of aluminum. *Environ Health Perspect* **65:** 363–441, 1986.
16. Buchet JP, Lauwerys R, Hassoun A, Dratwa M, Wens R, Collart F and Tielemans C, Effect of aluminum on porphyrin metabolism in hemodialyzed patients. *Nephron* **46:** 360–363, 1987.
17. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
18. Labbe RF, Porphyrins and related compounds. In: *Fundamentals of Clinical Chemistry* (Ed. Tietz NW), pp. 455–473, Saunders, Philadelphia, 1976.
19. Mauzerall D and Granick S, The occurrence and determination of δ-aminolevulic acid and porphobilinogen in urine. *J Biol Chem* **219:** 435–446, 1956.
20. Segel IH, *Enzyme Kinetics*. Wiley-Interscience, New York, 1975.
21. Astin KH, Bishop DF, Wetmur JG, Kaul B, Davidow B and Desnick RJ, δ-Aminolevulinic acid dehydratase isozymes and lead toxicity. *Ann NY Acad Sci* **514:** 23–29, 1987.
22. Abraham NG, Levere RD and Freedman ML, Effect of age on rat liver heme and drug metabolism. *Exp Gerontol* **20:** 277–281, 1985.
23. Birchall JD and Chappell JS, Aluminum, chemical physiology and Alzheimer's disease. *Lancet* **ii:** 1008–1010, 1988.
24. Martin RB, The chemistry of aluminum as related to biology and medicine. *Clin Chem* **12:** 1797–1806, 1986.
25. Jaffe EK, Salowe SP, Chen NT and DeHaven PA, Porphobilinogen synthase modification with methylmethanethiosulfonate. *J Biol Chem* **259:** 5032–5036, 1984.
26. Jaffe EK and Hanes D, Dissection of the early steps in the porphobilinogen synthase catalyzed reaction. *J Biol Chem* **261:** 9348–9353, 1986.