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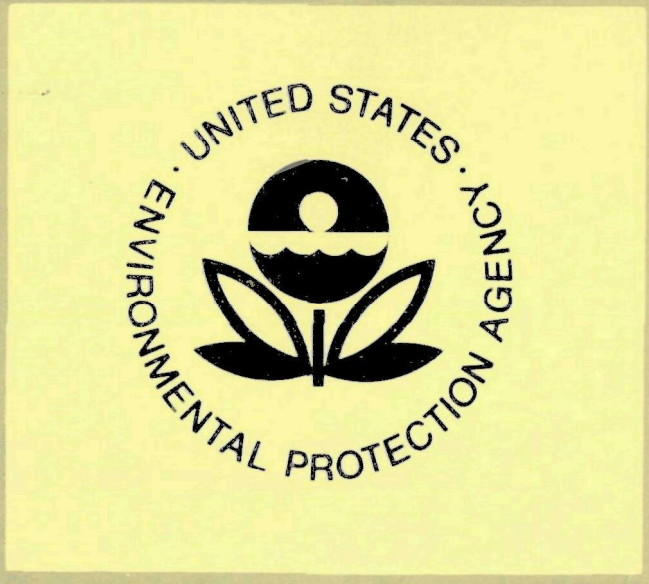
Environmental Health Effects Research Series

EFFECT OF LEAD ON GAMMA AMINO BUTYRIC ACID SYNTHESIS



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EFFECT OF LEAD ON GAMMA AMINO BUTYRIC ACID SYNTHESIS

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Research pertaining to gamma amino butyric acid and glutamic amino acid decarboxylase is especially important because there is yet a paucity of information concerning the specific neurochemical function of this enzyme system and how it may be affected by environmental insults. The results presented in this report showing inhibition of GADC activity by lead is significant to our further understanding of its neurotoxic effects in chronic conditions at the molecular level.

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ABSTRACT

This project was a study of the inhibitory effect of lead on the enzymatic activity of brain glutamic amino acid decarboxylase (GADC). The enzyme is responsible for the catalytic formation of gamma amino butyric acid (GABA) in inhibitory neurons, which is believed to be involved with the transmission of inhibitory impulses in the brain.

Lead nitrate solution was available to Sprague-Dawley female rats ad libitum and the quantity of lead ingested was determined by the volumes consumed. During the course of these experiments, animals were examined for weight loss, locomotor activity, excitability and other behavioral manifestations of lead toxicity. At autopsy, the tissues selected for lead determinations were brain, liver, bone (femur) and kidney. In another series of experiments, GADC was isolated from fresh bovine brain tissue and in vitro studies were performed to determine the nature of lead inhibition of the enzyme. Subsequently, Se and Cd inhibitions of the enzyme were studied and compared to lead inhibition. Light microscopic studies of liver, brain and kidney tissues were performed.

The activity of the enzyme GADC in brain tissue homogenates of rats drinking lead nitrate solutions was less than the activity determined in control rats. The Vmax of bovine brain GADC using glutamate as a substrate was 938 counts of $^{14}CO_2$ per minute in a system which contained 1.6×10^{-3} moles glutamate and tracer glutamate-1-^{14}C, (880,000 dpm) and the Km was 3.6×10^{-4} moles/liter. When the inhibiting effect of Pb, Cd, and Se on GADC activity were compared, Pb was the most potent inhibitor, Cd showed less inhibition and Se showed no inhibition of enzyme activity.

CONTENTS

List of Figures

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

AAS - atomic absorption spectrophotometry
B W - body weight
^{14}C - an isotope of carbon possessing radioactivity
Cd - cadmium
CM - centimeters
CNS - central nervous system
CO_2 - carbon dioxide
CPM - counts per minute
dpm - disintergrations per minute
g - gram
GABA- gamma amino butyric acid
GADC- glutamic acid decarboxylase
gms - grams
KOH - Potassium hydroxide
K_2HPO_4- potassium phosphate, dibasic
Km - Michaelis Constant
L - liter
M - molar concentration
m - mole
mls - milliliters
MSG - monosodium glutamate
Pb++- divalent lead ion
Pb - lead
POPOP- 1,4 bis -2-(5-phenyloxazoyl)-Benzene
PPO - 2,5 diphenyloxazole
S - substrate concentration
/ - per
u - micro
$\frac{1}{v}$ - reciporical of velocity or activity
$\frac{1}{s}$ - reciporical of substrate concentration
Vmax- maximum velocity

ACKNOWLEDGEMENTS

The cooperation of the Morrissey Meat Company, Nashville, Tennessee for providing fresh bovine brain is gratefully acknowledged. Assistance in the care of the experimental animals by the Animal Facilities Staff at Meharry Medical College is also appreciated.

The assistance given by Dr. Edward J. Faeder, former project officer, and Dr. Larry Hall, current project officer in the development and completion of this project is kindly acknowledged.

Section 1

INTRODUCTION

The occurrence of leading poisoning is perhaps one of the oldest occupational diseases known, and lead probably causes more poisonings than any other metal in the environment. Poisoning is not uncommon in children who reside in poor housing areas while adult lead poisoning is caused primarily by occupational exposure, by consumption of lead-contaminated whiskey or food,by beverages stored or served in improperly glazed earthenware, or by inhalation of the fumes resulting from battery factories.

When a small amount of lead is taken into the body from the enviroment, it is excreted via the kidney; however, when the intake is greatly increased, lead may accumulate to toxic levels in soft tissues and affect many organs of the body. The systemic effects may be appropriately placed under three headings; the CNS, the hematopoietic and renal systems.

Researchers who have studied lead toxicity differ on the lead plasma level that constitutes a toxic concentration, and there is considerable controversy with regard to whether the amount of lead with which modern man comes into contact is greater than that of his ancestors. It is a recent recognition that the absorption of lead by inhalation may be 10-20 times as great as absorption via the digestion tract or skin, and although lead poisoning has not disappeared, the clinical symptoms may not be immediately evident since absorption and accumulation to toxic levels in tissues may occur over an extended period of time.

This study has determined the level of orally administered lead sufficient to cause a measurable decrease in GADC activity in rat brains. It has also shown the effect of orally administered lead on the tissue absorption and the nature of the lead inhibiting of bovine brain GADC.

Section 2

CONCLUSIONS

There was no difference in the attrition rate of experimental animals which received up to one percent lead compared to control animals which received up to a one percent sodium nitrate solution ad libitum for sixteen weeks.

The average final weights of the experimental groups were slightly less than the control group, however the lead treatment had little effect on tissue or organ size except for the femur. The femur weighed less per one hundred grams of animal body weight after exposure to lead at both the 0.5 and 1.0 percent concentrations.

In animals exposed to 0.5 percent lead, no accumulation occurs in the liver and femur; however, there was a significant accumulation in the kidney, brain and whole blood, indicating selective tissue uptake of the heavy metal. At the one percent level the tissue with the greatest uptake per unit weight was the kidney, this tissue having levels nearly ten times higher than the brain, liver, femur and whole blood.

Assay of GADC activity of brain homogenates showed that as larger quantities of lead were consumed by the animals, correspondingly lower GADC activity was observed in homogenates prepared from their brains.

GADC isolated and purified from bovine brain can be used for model kinetic studies on competitive inhibition by lead.

Lead is the most potent inhibitor of GADC in comparison to Cd and Se.

Section 3

RECOMMENDATIONS

It is recommended that the pathophysiology at the molecular level of lead metabolism continue to be investigated and that the research efforts along these lines more definitively explain the relation of lead concentrations in various tissues with associated damage to the tissues.

Furthermore, it is recommended that there be continued basic research to determine how the presence of lead affects the metabolic response of animals to other toxic elements, and how the presence of disease states (sickle-cell anemia, emphysema, infection, and hypertension) may influence the response of the animal to various lead burdens.

It is recommended that a definitive study be done on a selected inner city population of children in which the total lead consumed from all sources (food, air) be monitored along with the total lead excreted. The subjects of this type of study should be evaluated during the course of the study using typical physical examination and evaluation procedures for determining behavioral changes. Families should be selected carefully so that attrition rates may be kept at a minimum and the community in which the study is to be made should be thoroughly apprised of the significance of the study. Utilizing schools, churches and community, civic, and social clubs could be of high value in this aspect of such a study.

Section 4

MATERIALS AND METHODS

Experimental animals, rats of the Sprague-Dawley strain, were obtained from the O'Fallion Laboratories, St. Louis, Missouri, at 21-23 days of age. They were housed individually in wire cages in the animal facility of Meharry Medical College during the entire course of the experiment, which began after a 25 day adaption and adjustment period. Hence, the rats were approximately 50 days old when the experiment started. During the course of the experiment, the rats were observed for physiological and behavioral symptoms of lead toxicity and the quantity of food and liquid consumption available ad libitum were recorded daily.

When the rats were killed, liver, blood, brain, kidney, and bone (femur) tissues were removed, weighed and a section placed in buffered formalin solution or immediately frozen for subsequent protein and lead analyses. GADC activity was assayed immediately in the brain of the animals using one hemisphere of the cerebral cortex for preparation of the homogenate (1,2). Protein was determined in all tissues using the Folin phenol protein nitrogen procedure (3). Lead was determined in samples that had been wet-ashed with HNO_3:$HClO_4$ using a Perkin Elmer Atomic Absorption Spectrophotometer Model 303 (4).

Fresh calf brains were contributed by Morrisey Meat Company, Nashville, Tennessee. GADC was isolated from calf brain following the procedure of Susz (2) with minor modifications. All centrifugations requiring a force greater than 600xg were done using a Beckman Model L-5-50 Ultra centrifuge and fractions from the Sephadex Columns were collected using Buchler Fractomette 200. Studies of the isolated enzyme were performed in vitro. Warburg Flasks were placed into a Bench Scale metabolic shaker and held at 37°C. The metal solutions were prepared from reagent grade pure metals by converting them to nitrate salts using HNO_3 and buffering with phosphate buffer to pH 6.5. For the assay of GADC activity the procedure of Lupein was followed (1). The liberated ^{14}C as CO_2 from the alpha carbon ^{14}C-labeled sodium glutamate (New England Nuclear) which was added to the homogenate was counted using a Beckman LS-150 Scintillation Counter. A PPO-POPOP soultion in Toluene (42 mls of Liquiflor in 1000 mls of toluene) was used as the scintillation fluid.

Section 5

EXPERIMENTAL PROCEDURES

GENERAL PROCEDURES

Experiment 1

Three different sets of rats were utilized in the study. In the first set, fifty day old rats of the Wistar strain were grouped into five groups with each group consisting of ten animals individually housed in wire cages. The lead was administered as the acetate salt in water which was available ad libitum. The concentrations of lead ranged from one to four percent with the control group receiving sodium acetate titrated with hydrochloric acid to pH of approximately 7.0. Many of these animals developed a respiratory problem, and the poor solubility of lead acetate in water encouraged us to switch to another strain and source of rats as well as lead nitrate which is more soluble in water. Remaining animals in this set were used by the research team for practice of the procedures that were to be subsequently carried out.

Experiment 2

The second set of animals (of the Sprague-Dawley strain) were divided into five groups, housed in a fashion identical to the first set but in improved animal quarters and observed for a period of six weeks. The five experimental groups had as the only source of liquid 1 percent Na NO_3 (control), 0.5 percent lead, 1 percent lead, 2 percent lead and 3 percent lead respectively, all as the nitrate salt. The average liquid consumed per week was tabulated from records of daily consumption, and the average amount of lead consumed per week was calculated per one hundred grams of body weight. During the six-week period, at weekly intervals, animals from each of the experimental groups were tested for glucosuria, righting reflex, locomotor activity, awareness and observed for skin ruptures, alopecia and hind leg paralysis.

Of all of these tests, only the observations of glucosuria, measured using dextrostix (Ames Laboratories, Elkhart, Indiana), skin rupture and alopecia were relatively free of bias. Awareness was assessed by counting the movements within a three minute time span across a central line drawn in the animal's cage. This parameter was measured only during the sixth week of the study.

Experiment 3

The third set of animals consisted of sixty albino rats of the Sprague-Dawley strain fifty days of age. They were divided into three groups consisting of twenty animals each. One group of animals received one per cent sodium nitrate as the only liquid source and served as the control group. A second group received a 0.5 percent lead solution as the nitrate as the only liquid source and the third group received a one percent lead solution as the nitrate as the only liquid source. Previous and preliminary studies had shown that nitrate administered to rats at the level comparable to the level of nitrate being consumed in the one per cent lead group was well tolerated. These animals were maintained for sixteen weeks.

The handling of all animals at the kill time and subsequent treatment of tissues were identical. Animals were weighed, placed under terminal ether anesthesia and a blood sample was drawn via cardiac puncture with a heparinized syringe. The liver, kidney, bone (femur) and brain were removed and weighed. One hemisphere of the brain was placed into buffered formalin. The other hemisphere was immediately homogenized, diluted to a volume of 10 mls with phosphate buffer, pH 7.4, and assayed for GADC activity. Lead and protein analyses were performed on aliquots of this homogenate. Representative samples of the livers, and kidneys, were placed into buffered formalin, and the remainder of the tissues frozen until lead and protein analyses could be performed.

The lead in all tissues was determined by atomic absorption spectroscopy using a Perkin-Elmer Model 303 with an air-acetylene flame. In this procedure, the known weights of tissue were wet-ashed using nitric acid, and after an overnight digest in Erlenmeyer flasks, perchloric acid was added to the samples as gentle heat was applied until a clear solution was obtained. Digested samples were quantitatively transferred to volumetric flasks and diluted to volume. When the lead level was too low to give reliable absorbance on the AAS 303, an aliquot of the sample solution was buffered to form a stable complex with ammonium pyrrolidine dithiocarbamate and the stable complex was extracted with methyl isbutylketone. This enhances the sensitivity of AAS for heavy metals.

Known aliquots of brain tissue homogenates were solubilized in hot KOH and protein determined. Brain hemispheres were removed intact by cutting the skull, weighed, and immediately placed into ice cold 0.02M phosphate buffer, pH 7.4. They were homogenized with a Potter-Elvejhem all glass homogenizer after the concentration was adjusted to one gram of tissue per 8.6 mls of buffer.

From this homogenate, 5 mls were pipetted into the outside chamber of Warburg Flask into which 20 ul of glutamic acid-1-^{14}C (880,000 dpm) and 0.7 ml of 0.4 per cent non radioactive sodium glutamate had been previously introduced. They flasks were gently agitated to assure mixing of the contents, and 0.24 ml of 16N H_2SO_4 were placed into the side arm of the flask.

A multifolded strip of Whatman No. 1 filter paper (3 x 5 cm) which had been saturated with Hymine hydroxide were placed into the center well of the flasks, the flasks gently flushed with nitrogen, capped with a glass stopper and placed into the metabolic shaker which contained water at 37°C.

At the end of a thirty minute incubation period the H_2SO_4 was emptied from the side arm into the reaction chamber of the flask in order to stop the reaction. The flasks were than reincubated for an additional fifteen minutes at 37°C. This enabled the HCO_3^- in the solution which formed upon the acidification by the H_2SO_4, to be converted to CO_2. The CO_2 was trapped onto the Hymine hydroxide impregnated filter paper. The filter paper was then placed into a scintillation counting vial which contained 15 mls of scintillation counting solution. The vials were counted for radioactivity after standing for a minimum of eight hours.

Histologic slides were prepared using the routine hematoxylin and eosin stains and observed under the light microscope for evidence of nuclear damage and cellular necrosis.

The calf brains form which the GADC was isolated were obtained from a local supplier minutes after the animals was killed. They were immediately brought to the laboratory and the isolation procedure initiated as detailed by the flow digram in Figure 2.

The Supernatant IV (Figure 2) was placed on a 45 x 2.5 cm column and fractions of eluant collected in three ml aliquots. Absorbance was measured at 280 nM using a Beckman Model 25 spectrophotometer, and assays for GADC carried out on selected tubes. The GADC rich fractions were pooled, lyophylized, and stored in the forzen state in a buffer containing glutatione and pyridoxal phosphate each at a concentration of 10^{-4} moles/liter. An aliquot of this preparation was subjected to electrophoresis using a Beckman Microzone System with a cellulose acetate membrane, pH 8.6.

Section 6

RESULTS AND DISCUSSION

Animal Studies

Data collected from rats in experiment two is summarized in Tables 1 and 2. In Table 1, information is presented showing the toxicity of lead nitrate. When the lead as the nitrate was available in the drinking water at 2 grams percent or above, death occurred within two weeks for twenty-eight of thirty female rats of the Sprague-Dawley strain. The consumption of lead solution was less by the experimental groups when compared to the nitrate control group over the six week experimental period. Rats receiving 0.5 percent lead solution and those receiving one percent lead solution gained weight. Those receiving 2 percent lead lost nearly half of their initial weight before dying, and those receiving 3 percent lead died within three days without any weight loss. Only two of fifteen animals receiving the 2 percent lead survived for the six week period.

With reference to the growth data, the averages of the initial weights of the nitrate controls, the 0.5 percent test group and the one percent test group, suggest that the lead might abate the slight growth supression effect of the nitrate. The environmental implication of this observation merits further inquiry. Heavy metals presented to the atmosphere are generally accompained by the release of nitrates and sulfates or the oxides which may be converted to various nitrogen and sulfur oxygen containing anions. Such anion production might have the overall effect or reducing environmental lead toxicity. To be sure, this is highly speculative and does not negate the environmental impact of these anions as bio-hazardous substances.

The parameters observed in the animals were glucosuria, presence of righting reflex, skin rupture, alopecia, locomotor activity and awareness. The observations were made on the animals at weekly intervals and no significance could be attached to any of the data except the awareness. Animals receiving the one percent lead were less aware than the nitrate control groups. This parameter was measured by counting the number of times the animals crossed a line drawn on the bottom center of the cage. The number of movements across the line per three minute periods were recorded and the average calculated. Seven animals from each group were utilized. The number of movements for the nitrate control group was 4.8

per three minute time period, while the group receiving 0.5 per cent lead crossed the line an average of 5 times and the group receiving one per cent lead crossed the line an average of 2.6 times. This implies that the group receiving one per cent lead was less aware than the other groups.

The GADC activity observed in brain homogenates was somewhat inversely related to the quantity of lead in the solutions that the animals had received. The findings are summarized in Table 2. The evidence shows that lead accumulated in the brain tissue when given at the 0.5 per cent level or any level.

In the third experiment, rats were placed on the experiment at fifty days of age. The average amount of lead consumed (recorded in grams of lead per 100 grams of animal weight over a sixteen week period as calculated from the volume of lead solution consumed) fluctuated in a much more pronounced manner for the one per cent lead group than for the 0.5 per cent lead group. The pattern is presented in Figure 1. Also, as the time of the experiment progressed, the lead intake for the one per cent group generally decreased such that after sixteen weeks on the experiment the average lead consumption was down to approximately thirty-five per cent (0.31 g Pb/week/100 gms) of the high of 0.86 gm Pb/week/100 grams.

The attrition schedule was acceptable as deaths were not limited to any single experimental group and the deaths of the rats occurred throughout the entire course of the study. Table 3 gives the attrition schedule.

The growth rate depression effect of lead is seen in Table 4. While there was wide variation in the final weights of animals within the epxerimental groups, the trend observed was that the group receiving the one per cent lead weighed less than the other two group.

In Table 5, the results were presented showing the effect of lead on the weights of the liver, kidney, femur and brain. The weights of these tissues are expressed as grams per one hundred grams of animal weight. No significant differences were observed for any of the tissues studied, except the general trend suggests that a smaller femur resulted from the lead consumption.

Table 6 shows the distribution of lead in these tissues. As expected, the blood lead levels were higher in the one per cent lead group. While the lead concentration was only twice as high in the liquid of this group as compared to the 0.5 per cent group, the lead level in the blood in the one per cent lead group was three times as high (77 ug%) as the level in the 0.5 per cent group (23 ug%). Some lead was found in the blood and all other tissues of the control group. This lead is no doubt of dietary orgin. (Purina Laboratory Chow which the animals were fed contained 34 ug Pb per gram). The femur of the control group contained as much lead as did the 0.5 per cent lead test group and only slightly less than the one per cent lead test group. (28, 28 and 33 ugs Pb/gram dry weight, respectively). This finding suggests that there was adequate lead in the animal feed to saturated the capacity of the bone to store lead. Further

support of the finding is the increased levels of lead found in the brain and kidney tissues of both test groups. Only the one per cent lead group showed accumulated lead in the liver. The lead level in the kidney probably represents an accumulation associated with attempts to excrete as well as store it, but once the renal capacity is surpassed, the storage and excretion via the liver occurs. Of the three soft tissues analyzed for lead, brain and kidney accumulated significant amounts of lead in the 0.5 per cent grams but liver did not. In the one per cent group, the kidney contained more lead than did the kidney of animals from the 0.5 percent lead group. These findings suggest that brain and kidney are among the soft tissues capable of accumulating lead at low consumption levels if the rate of consumption is high enough to elevate blood lead significantly.

When the brain homogenates of the rats were assayed for GADC activity and the protein content of the homogenate determined, it was observed that the activity of the enzyme was less in homogenates of rats given the higher lead solutions. These findings are presented in Table 7.

Enzyme Studies

The procedure of Susz et al, (2) was used for isolation of the enzyme from calf brain in a 500-fold purification. This value was determined by measuring the activity of the enzyme at different stages of isolation and purification beginning with the brain homogenates and expressing the enzyme activity as the radioactive ^{14}C liberated per unit weight of protein. The homogeneity of the isolated protein was assessed using Microzone electrophoresis, pH 8.6, with cellulose acetate membranes as the support system. The homogeneity, it was concluded, was sufficient such that the protein could be used for enzyme studies. Figure 3 shows the electrophoretic pattern obtained using this system and comparing it with a control specimen. Using the data in Table 8 the Vmax and Km of the enzyme preparation was claculated. Based on data presented in Table 8 the Vmax was 1034 counts per minute per microgram of protein and the Km obtained by extrapolation was 4.5×10^{-4} moles of glutamic acid per milliliter. When the first seven data points were placed on the Sigma 7 Computer and the Vmax and Km determined by use of a program first adapted by W. W. Cleland, the Vmax was determined to be 938 CPM per microgram of protein and the Km was 3.6×10^{-4} moles of glutamic acid per ml. Susz (2) reported that the Km of the GADC isolated from rat brain to be 3.9×10^{-3} moles of glutamic acid per liter. The difference may be due to species, since bovine brain was used in this study, and/or the differences may be due to different degrees of purity of the enzyme preparation.

In Table 9 the results showing the effect of lead on calf brain GADC activity *in vitro* is given. It is noted that substrate inhibition of the enzyme occurs as suggested by the data in column I; however, at all substrate concentrations below 2.01×10^{-8} moles per liter, 10^{-4} mole of Pb of lead inhibited the enzyme. It may be that the higher lead concentrations complex with the substrate making less lead and substrate available to inhibit the enzyme.

An experiment designed to show the comparative inhibitory effects of Pb, Cd and Se on GADC activity *in vitro* showed that Pb is the most potent inhibitor of the three metals and Se the least potent. Table 10 give the per cent inhibition of the three metals on the enzyme.

The data presented indicates that *in vitro* lead inhibits GADC and that this inhibition may be overcome by addition of substrate. Such reversal of inhibition is expected for competitive inhibitors.

This study has shown that the levels of the activity of the enzyme, GADC which may be detected in rat brain homogenates given sub-lethal levels of lead in their drinking water is decreased. This may be associated with decreased synthesis of GABA; however, the quantitation of brain GABA in the brains of animals so treated was not done.

Many researchers have shown associations of lead with disturbed CNS function and amine metabolism (7,8,9, 10), and the role of GABA in synaptic transmission in the CNS seems to be clearly established (11, 12).

One of the clinical symptoms of acute lead toxicity is the onset of convulsions. These convulsions may be terminated by the use of complexing agents as penicillamine and/or versene (13). We speculate that the removal of the lead by these complexing agents results in reactivated GADC and as GABA is endogenously formed, the convulsions cease.

Apparently in gradual depletion of GADC activity, there are no convulsions. Such has been the observation of Bayoumi *et. al* (14). They showed that in the brains of rats placed on B_6 deficient diets and a B_6 antagonist (4-deoxypyridoxine), while both groups of animals had lower GADC activity, no convulsions occurred. This may be explained by the fact that GABA did not get to some critically depleted level, or that other neuroinhibitory transmitter substances may have been produced which protected the animals from convulsions.

REFERENCES

1. Lupein, P. J., C.M. Hinse and L. Berlinguet. Determination of Glutamic Acid Decarboxylase Activity in Rat Brain. Analytical Biochemistry 24: 1-8, 1968.

2. Susz, Jean P., Bernard Haber and Eugene Roberts. Purification and Some Properties of Mouse Brain L-Glutamic Acid Decarboxylase. Biochemistry 5: 2870-2877, 1966.

3. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R. J. Randall: Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 265-275, 1951.

4. The Perkin-Elmer Corporation. Analytical Methods for Atomic Absorption Spectrophotometry. Lead Standard Conditions and Lead in Blood. 1968.

5. Proskey, Leon and R. G. O'Dell. Effect of Dietary Monosodium L-Glutamate on Some Brain and Liver Metabolites in Rats. (35930). P.S.E.B.M: 138 517-522, 1971.

6. Roberts, Eugene, and Sam Frankel. -Amino Butyric in Brain: its Formation from Glutamic Acid. J. Biol. Chem: 187, 55-63, 1950.

7. Goyer, R. A. and B.C. Rhyne. Pathological Effects of Lead. Int. Rev. Exp. Path 12:1-77, 1973.

8. Thomas, J. A., F.D. Dallenbach, and M. Thomas. Considerations on the Development of Experimental Lead Encephalopathy Virchows Arch. (Pathol. Anat): 352: 61-74, 1971.

9. Souerphoff, Mitchell W. and I. A. Michaelson. Hyperactivity and Brain Catecholamines in Lead-Exposed Developing Rats. Science 182: 1022-1024, 1973.

10. Golter, M. and I. A. Michaelson. Growth Behavior and Brain Catecholamines in Lead-Exposed Neonatal Rats: A Reappraisal. Science 187: 359-361, 1975.

11. Roberts, Eugene and K. Kurimaya. Biochemical-Physiological Correlations in Studies of the Gamma-Aminobutyric Acid System. Brain Research 8: 1-35, 1968.

12. Krnjevic, K. Micro-Ionophoretic Studies on Cortical Neurons. Int. Rev. Neurobiol: 7:41-98, 1964.

13. Chisolm, J. J. and E. Kaplan. Lead Poisoning in childhood. Comprehensive Management and Prevention. J. Pediatr. 73: 942-950, 1968.

14. Bayoumi, R.A., J. R. Kirwan and W.R. Smith. Some Effects of Dietary Vitamin B_6 Deficiency and 4-Deoxypyridoxine on -Amino butyric Acid Metabolism in Rat Brain. Journal of Neurochemistry: 19: 569-576, 1972.

BIBLIOGRAPHY

1. Carson, T.L., Gary A. Van Gelder, George C. Karas, and William B. Buck. Slowed Learning in Lambs Prenatally Exposed to Lead. Arch. of Environ Health 29: 154-156, 1974.

2. Haber, Bernard, K. Kyriyama, and E. Roberts. Mitochondrial Localization of a New L-Glutamic Acil Decarboxylase in Mouse and Human Brain. Brain Research 22: 105-112, 1970.

3. Patel, A.J., I.A. Michaelson, J.E. Cremer and R. Balazs. Changes within Metabolic Compartments in the Brains of Young Rats Ingesting Lead. Journal of Neurochemistry. 22: 591-598, 1974.

4. Sauer, R.M., B.C. Zook, and F.M. Garner. Demyelinating Encephalomyelopathy Associated with Lead Poisoning in Nonhuman Primates. Science: 169, 1091-1093, 1970.

5. Tapai, R., Pasantes Herminia, and G. Massieu. Some Properties of Glutamic Acid Decarboxylase and the Content of Pyridoxal Phosphate in Brains of Three Vertebrate Species. Journal of Neurochemistry: 17, 921-925, 1970.

6. The Medical Clinics of North America. Trace Elements. 1976. Vol. 60, No. 4 W. B. Saunders Company, Philadelphia, Pa. 19105. Albert M. Meier, Editor.

TABLE 1. EFFECT OF LEAD ON THE WEIGHT OF RATS

Group	Number of Animals	Average Volume Liquid Consumed/week (ml)	Average Pb Consumed *	Average g Beginning Wt	Average g Terminal Wt
Control	10	157	0	181	186
0.5% Pb	15	145	0.42	202	228
1.0% Pb	10	137	1.29	189	243
2.0% Pb	15	#	-	182	103
3.0% Pb	15	##	-	163	163

* In grams per week per 100 g body weight

Thirteen animals expired within two weeks.

All animals expired within one week.

- No data collected.

TABLE 2. GADAC ACTIVITY IN RAT BRAIN HOMOGENATES MEASURED AS cpm $^{14}CO_2$ RELEASED

Group	CPM/gram Brain
1.0% (14)*	3114
0.5% (13)*	2225
1.0% (11)*	1915
2.0% (3)*	947

* Number of animals in each group. Animals sacrificed after six weeks.

TABLE 3. ATTRITION SCHEDULE OF ANIMALS RECEIVING LEAD AS LEAD NITRATE

	WEEK																
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0.0%Pb	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	4
0.5%Pb	2	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	5
1.0%Pb	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	4
TOTAL	3	0	0	0	0	1	2	0	0	1	0	0	1	2	1	0	13

TABLE 4. THE EFFECT OF LEAD THE WEIGHT OF RATS

Group	Volume Liquid Consumed/Week (mls)	Average Lead Consumed*	Initial Weight (g)	Terminal Weight-16 wks (g)
1.0% NO_3 as $NaNO_3$	133	-	71 ± 6	166 ± 24
0.5%Pb	85	0.18 ± .003	79 ± 5	155 ± 27
1.0%PB	83	0.47 ± .22	80 ± 5	146 ± 37

* In grams per week per 100g body weight.

TABLE 5. EFFECT OF LEAD ON ANIMAL TISSUE WEIGHT

Group	Sacrifice Weight (Gms)*	LIVER#	KIDNEY#	FEMUR (DRY)#	BRAIN#
1% $NaNO_3$	166 ± 24	3.4 ± 0.7	1.1 ± 0.2	0.56 ± .02	1.2 ± 0.4
0.5% Pb	155 ± 27	2.8 ± 0.6	1.3 ± 0.2	0.46 ± .04	1.1 ± 0.2
1.0% Pb	146 ± 37	3.0 ± 0.6	1.2 ± 0.4	0.44 ± .03	1.1 ± 0.3

* After 16 weeks

Expressed as gms/100 gms animal weight

TABLE 6. CONCENTRATION OF LEAD IN BLOOD AND TISSUES

Group	Whole Blood*	TISSUE (ug Pb/g)			
		Femur	Brain	Kidney	Liver
1.0% $NaNO_3$	9 ± 0.01	28 ± 1.2	0.36	0.4 ± 1.4	0.07 ± 0.01
0.5% Pb	23 ± 0.02	28 ± 5.6	2.0	18 ± 5.0	0.08 ± 0.06
1.0% Pb	74 ± 0.03	33 ± 6.8	1.7	25 ± 2.0	0.52 ± 0.07

*Micrograms Pb per 100 mls blood.

TABLE 7. GADC ACTIVITY IN RAT BRAIN HOMOGENATES MEASURED AS CPM $^{14}CO_2$ RELEASED

Group	CPM/gram Brain	Pb ug/gram Brain	CPM/ug Pb
1.0% $NaNO_3$	3403	0.36	9453
0.5% Pb as NO_3	2177	2.0	1089
1.0% Pb as NO_3	1750	1.7	1029
2.0% Pb as NO_3*	1169	1.3	899

* Only two animals. All other data represent the average of six animals.

TABLE 8. KINETIC STUDIES ON CALF BRAIN GADC IN VITRO

$\times 10^{-4}$ M/Liter $\frac{1}{S}$		ACTIVITY $\left(\frac{1}{V}\right)$	
S	$\frac{1}{S}$	CPM*	1/CPM
.200 =	5.0	304.52 =	0.003.0
.400 =	2.5	495.58 =	0.002.0
.900 =	1.1	707.81 =	0.001.4
1.900 =	0.5	801.09 =	0.001.2
3.800 =	0.3	822.31 =	0.001.2
5.700 =	0.2	869.68 =	0.001.1
9.500 =	.14	919.06 =	0.001.0
11.400 =	.08	661.86 =	0.001.5
13.300 =	.08	541.15 =	0.001.8

* CPM per microgram of enzyme protein

TABLE 9. EFFECT OF LEAD ON CALF BRAIN GADC ACTIVITY IN VITRO

(S) x 10^{-8} mole/liter	CONTROL CPM/mg Protein x 10^2	10^{-4}M Pb CPM/mg Protein x 10^2
0.134	12.70	1.77
0.330	11.74	0.32
0.670	5.70	0.60
1.340	3.58	0.62
2.010	0.30	6.15
2.680	----	19.23
	Background 16 cpm	Background 16 cpm

* [1]Each reaction chamber contained 26 micrograms of purified enzyme protein preparation.

TABLE 10. COMPARATIVE INHIBITION EFFECT OF LEAD, CADMIUM AND SELENIUM ON GADC ACTIVITY

	Percent Inhibition		
Concentration	Pb*	Cd*	Se*
2×10^{-2}M	61	45	3
2×10^{-3}M	42	27	10
2×10^{-4}M	31	17	8
2×10^{-5}M	24	0	2
0	-	-	-

* Prepared as Nitrates and Buffered to pH 7.4 with 0.02 M Phosphate

Figure 1. AVERAGE LEAD CONSUMPTION DURING EXPERIMENTAL PERIOD

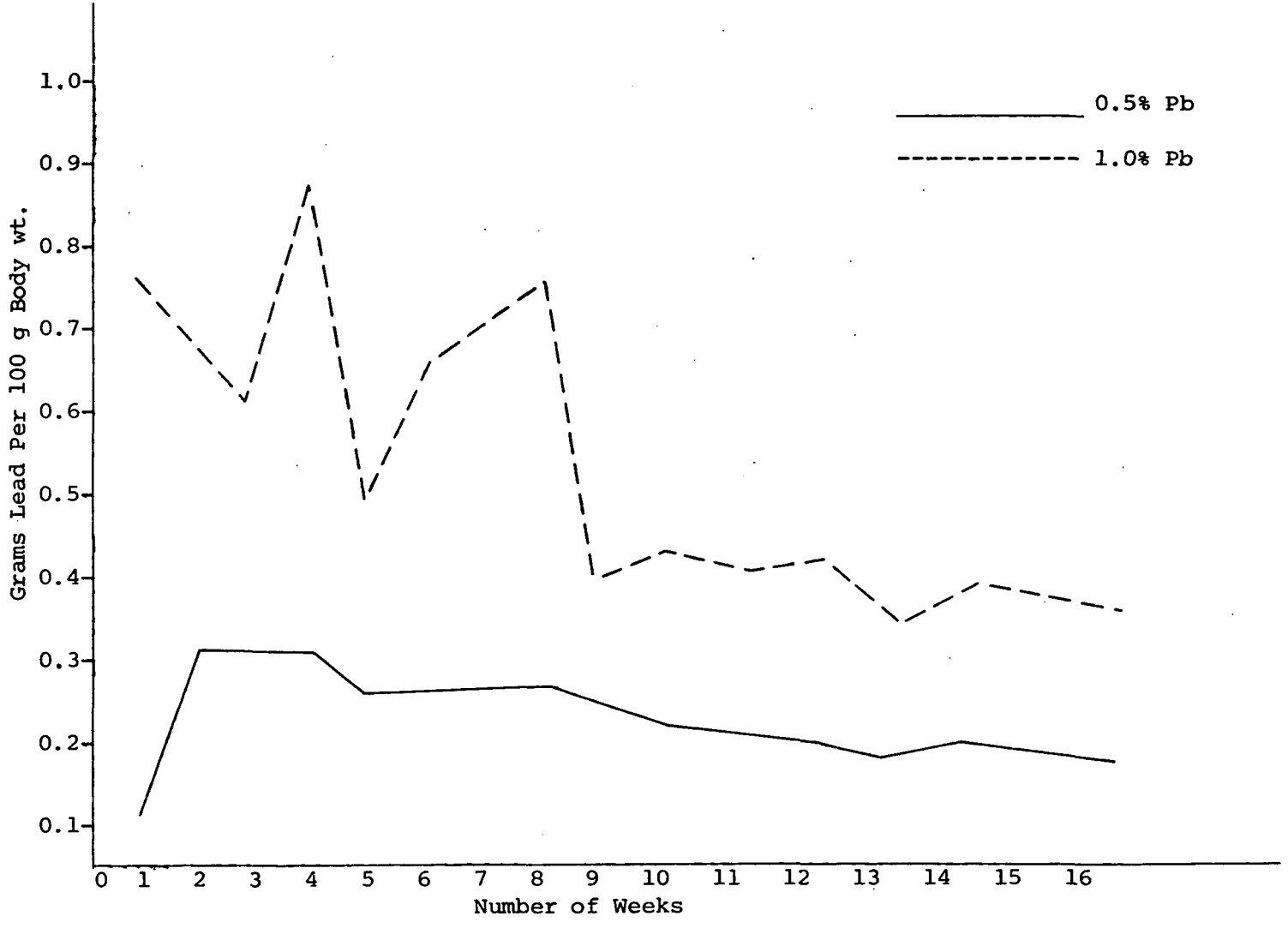


Figure 2. GADC ISOLATION AND PURIFICATION

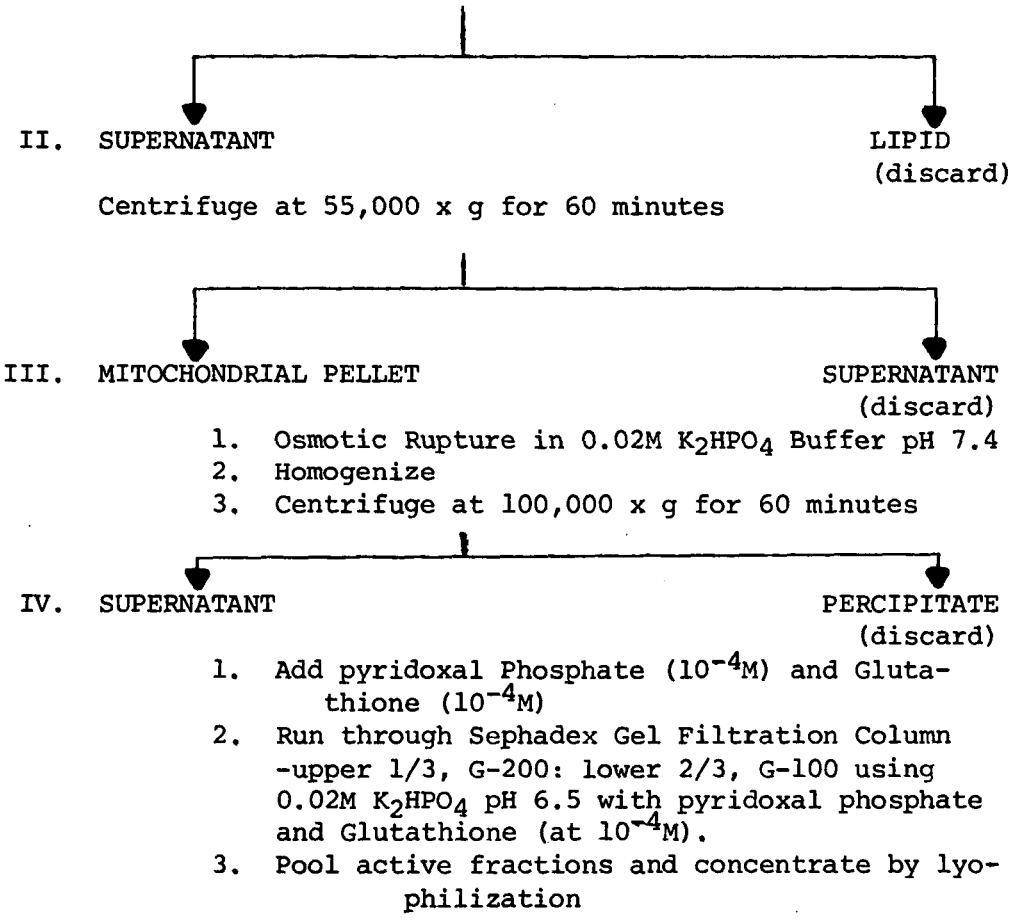
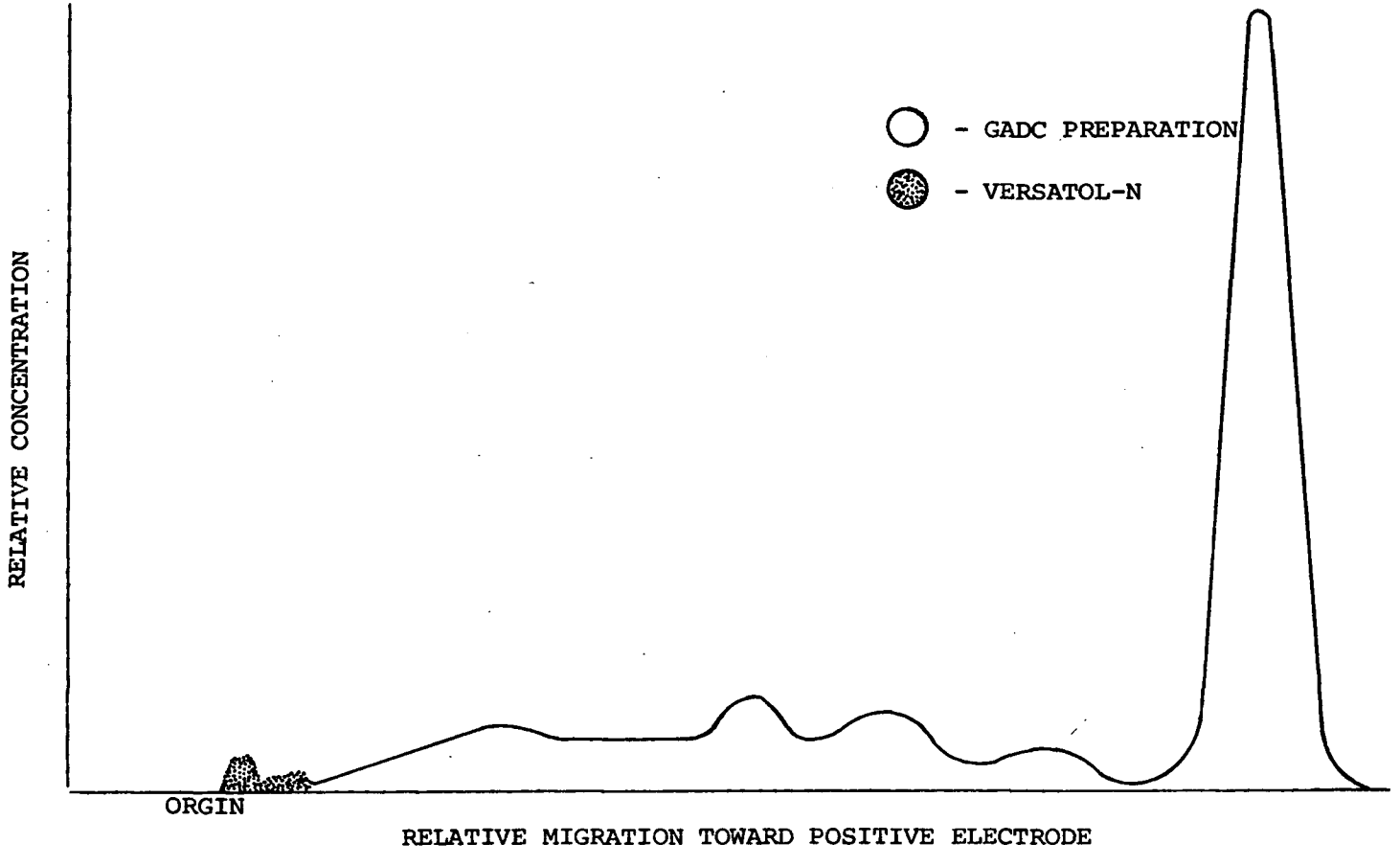


Figure 3. ELECTROPHORETIC PATTERN OF GADC PREPARATION COMPARED TO A VERSATOL-N CONTROL



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15. SUPPLEMENTARY NOTES

16. ABSTRACT

This project was a study of the inhibitory effect of lead on the enzymatic activity of brain Glutamic Amino Acid Decarboxylase (GADC). The enzyme is responsible for the catalytic formation of gamma amino butyric acid (GABA) inhibitory neurons which is believed to be involved with the transmission of inhibitory impluses in the brain.

Lead nitrate solution was available to Sprague-Dawley female rats ad libitum and the quantity of lead ingested was determined by the volumes consumed. During the course of these experiments, animals were examined for weight loss, activity, and excitability and other behavioral manifestations of lead toxicity. At autopsy, the tissues selected for lead determinations were brain, liver, bone (femur). In another series of experiments GADC was isolated from fresh bovine brain tissue, and in vitro studies were performed to determine the nature of lead inhibition of the enzyme. Subsequently, Se and Cd inhibition of the enzyme were studied and compared to lead inhibition. Light microscopic studies of liver, brain and kidney tissues were performed.

The activity of the enzyme GADC in brain tissue homogenates of rats drinking lead nitrate solutions was less than the activity determined in control rats. The Vmax of bovine brain GADC using Glutamate as a substrate was 1.54×10^{5} and the Km was 3.2×10^{-4} moles/liter. When the inbibiting effect of Pb, Cd, and Se on GADC activity were compared, Pb proved to be the most potent inhibitor, while Cd showed less inhibition, showed no inhibition of enzyme activity.

17. KEY WORDS AND DOCUMENT ANALYSIS

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