Histochemical Demonstration of the Alterations in the Renal Dehydrogenases Activities Induced by Lead in Wistar Albino Rats (*Rattus norvegicus*)

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A total of 112 male albino rats (*Rattus norvegicus*) were divided into seven groups and exposed to lead acetate trihydrate (0, 0.125, 0.25, 0.5, 1.0, 2.0 and 4 per cent for 1 to 8 months) in drinking water to investigate possible histochemical changes of nine renal dehydrogenases due to lead intoxication. A marked increase in the activity of lactate-, glucose-6-phosphate-, α-glycerophosphate-, reduced nicotinamide adenine dinucleotide (NAD)- and reduced nicotinamide adenine dinucleotide phosphate (NADP) dehydrogenase was observed while significant reduction was recorded in the activity of succinate-, malate-, isocitrate- and glutamate dehydrogenase. Changes in the activity of renal dehydrogenases were seen mainly in the pars recta and to a lesser extent in the pars convoluta and the ascending thick segment of the loop of Henle while the other medullary portions of the renal tubule were less affected. These histochemical findings led us to conclude that such changes in the renal dehydrogenases activities were due to chronic lead exposure and could be an adaptation to the metabolic, structural and functional alterations in the organelles of the renal cells especially the mitochondria.

Key words —— lead, dehydrogenase, kidney, rat, histochemistry

INTRODUCTION

Lead intoxication is probably the most common form of heavy metal intoxication[1–3] and is well documented as one of the most dangerous and insidous poisons to man.[4–7] The absorbed lead is conjugatad in the liver and is passed to the kidney, where a small quantity is excreted and the rest accumulates in the body.[8] Impaired kidney functions have been reported as one of the most silent feature of lead toxicity.[9–11] A considerable number of biochemical and enzymatic studies have been carried out on the effect of lead poisoning on the renal dehydrogenases activities.[6,12–18] Biochemical techniques are not sufficient in the study of the kidney where cell types comprising the tubular epithelium are heterogeneous. In addition little histochemical evaluation of the renal dehydrogenases was carried on the effects of chronic subtoxic exposure to lead. Therefore, the purpose of the present study is to investigate the histochemical alterations of renal dehydrogenases activities following experimental lead poisoning of Wistar albino rats by subtoxic doses of lead acetate trihydrate as one of the initial events responsible for further impairment to renal tissue function.

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MATERIALS AND METHODS

Animal and Housing —— A total of 112 male Wistar albino rats (*Rattus norvegicus*) of the same age weighing 110–130 gm of the King Saud University colony were used. Animals were randomly devided into seven groups of 16 rats, caged at room temperature and received food and water *ad libatum*.

Treatment —— Following a period of stabilization (7 days), lead acetate trihydrate was administered in drinking water at the rate of 0.0, 0.125, 0.25, 0.5, 1.0, 2.0 and 4%. Lead acetate was made soluble in water by addition of 1–2 drops of acetic acid. The rats were maintained on standard laboratory animal diet pellets (Grain Silos and Flour Mils Organization, Riyadh). Two animals from each group were killed by dislocation of the neck after 1, 2, 3, 4, 5, 6, 7 and 8 months of treatment. The total body, kidneys and liver of each animal were separetely

weighted.

Tissue Processing —— Portions of fresh kidney (cortex and medulla) from each rat were cut out rapidly, frozen in liquid nitrogen and stored in air tight tubes at –80°C until use. Cryostat sections (8–10 μm thick) were cut at –25°C.

Histochemical Characterization —— Dehydrogenases were histochemicaly demonstrated in the present study by using tetrazolium techniques.[19–20] The final incubation medium for each enzyme consisted of the nitro blue tetrazolium (NBT) stock solution, the substrate solution and the employing coenzyme. The NBT stock solution consisted of 2.5 ml NBT solution (4 mg/ml), 2.5 ml of 0.2 M Tris buffer (pH 7.4), 1 ml of 0.05 M magnesium chloride and 3 ml of distilled water.[21,22] Potassium cyanide (100 mM) or sodium azide (100 mM), a respiratory chain inhibitors of cytochrome systems, was added to the final incubating medium of the bound dehydrogenases. Phenazine methosulphate, an intermediate electron acceptor was added to the incubating media of the soluble dehydrogenases in the rate of 1 mg/ml of the medium.[23,24]

The activities of the soluble nicotinamide adenine dinucleotide-dependent dehydrogenases were demonstrated by means of the optimizid polyvinyl alcohol technique,[25] where 2 ml of 20 per cent polyvinyl alcohol were mixed with 2 ml of incubation medium of the dehydrogenases.

Histochemical reactions for dehydrogenases were performed on fresh unfixed cryostat sections. Prior to carrying out the reactions, sections were treated with cold acetone (± 0°C for 5 min) in order to remove lipids.[26,27] Lipid extracted unfixed fresh frozen cryostat sections were used for the histochemical characterization of renal dehydrogenases according to the following methods:

Succinate Dehydrogenase (SDH) —— This enzyme was demonstrated according to Lojda *et al*. and Frederiks *et al*. methods.[21,28] The final incubating medium consisted of 9 ml of NBT tetrazolium stock solution, 1 ml of 1 M disodium succinate (0.675 gm/ml), 2 drops of 0.5 per cent menadione dissolved in acetone. The pH of the medium was adjusted to 7.4. Air-dried 8 μm cryostat sections were incubated at 37°C for 30 min in the dark, post-treated in 4 per cent formaldehyde for 10 min and mounted in glycerine jelly. Control sections were incubated without succinate.

α-Glycerophosphate Dehydrogenase (αGPDH) —— The reaction revealing αGPDH was based on Frederiks *et al*. method.[28] The incubation medium of αGPDH consisted of 9 ml of NBT tetrazolium stock solution, 1 ml of 1 M disodium α-glycerol phosphate solution (0.315 gm/ml) and 0.1 ml acetone containing 1 mg menadione. The pH of the medium was adjusted to 7.3. Sections were incubated at 37°C for 45 min, post-treated in 4 per cent formaldehyde and mounted in glycerine jelly. Control sections were incubated in the absence of disodium α-glycerol phosphate.

Lactate Dehydrogenase (LDH) —— This enzyme was demonstrated according to Bancroft -Stevens and Frederiks *et al*. methods.[22,29] The final incubating medium consisted of 9 ml NBT tetrazolium stock solution, 1 ml of 1 M sodium DL-lactate (0.125 ml/ml) and 2 mg nicotinamide adenine dinucleotide (NAD). The pH of the medium was adjusted to 7.3. Sections were post-treated in 4 per cent formaldehyde and mounted in glycerine jelly.

Glucose-6-Phosphate Dehydrogenase (G6PDH) —— The activity of this enzyme was demonstrated according to Van Noorden.[30] The final incubating medium consisted of 9 ml NBT tetrazolium stock solution, 1 ml of 1 M disodium glucose-6-phosphate (0.3 gm/ml), 2 mg nicotinamide adenine dinucleotide phosphate (NADP), 0.1 ml sodium azide (100 mM) and 5 mg phenazine methosulphate. The pH of the solution was adjusted to 7.4. Sections were incubated for 20 min at 37°C, post-treated in 4 per cent formaldehyde and mounted in glycerine jelly. Control sections were incubated in the absence of disodium glucose-6-phosphate or NADP.

Malate Dehydrogenase (MDH) —— The reaction revealing MDH was based on Lojda *et al*. and Van Noorden-Frederiks methods.[21,31] The final incubating medium consisted of 9 ml of NBT tetrazolium stock solution, 1 ml of 1 M malic acid (0.134 gm/ml) and 2 mg NAD. The pH of the solution was adjusted to 7.5 with 40 per cent NaOH. Sections were incubated at 37°C for 60 min, post-treated in 4 per cent formaldehyde and mounted in glycerine jelly. Control sections were incubated without malic acid or NAD.

Isocitrate Dehydrogenase (ICDH) —— The activity of ICDH was performed according to Van Noorden and Frederiks method.[31] The incubation medium consisted of 9 ml of NBT tetrazolium stock solution, 1 ml of 1 M trisodium DL-isocitrate (0.27 gm/ml), 2 mg NAD and 5 mg phenazine methoslphate. The pH of the solution was adjusted to 7.4. Sections were incubated at 37°C for 15 min, post-treated in 4 per cent formaldehyde and mounted in glycerine jelly. Control sections were incubated

without isocitrate or NAD.

Glutamate Dehydrogenase (GDH) —— The activity of this enzyme was demonstrated according to Bancroft and Stevens method.[22] The final incubating medium consisted of 9 ml of NBT tetrazolium stock solution, 1 ml of 1 M sodium glutamate (0.187 gm/ml) and 2 mg NAD and 5 mg phenazine methosulphate. Sections were incubated at 37°C for 30 min, post-treated in 4 per cent formaldehyde and mounted in glycerine jelly. Control sections were incubated without sodium glutamate or NAD.

NADH Dehydrogenase —— This enzyme was demonstrated by the method of Rieder *et al*.[25] as modified by Bancroft and Stevens.[22] The incubating medium consisted of 9 ml of NBT tetrazolium stock solution, 1 ml distilled water, 2 mg NADH and 0.1 ml sodium azide (100 mM). The pH of the solution was adjusted at 7.4 and the solution preheated to 37°C. Sections were incubated at 37°C for 15 min, post-treated in 4 per cent formaldehyde and mounted in glycerine jelly.

NADPH Dehydrogenase —— The reaction of this reductase was performed by the method of Pearse as modified by Straatsburg *et al*.[24] The final incubating medium consisted of 9 ml NBT tetrazolium stock solution, 1 ml distilled water, 2 mg NADPH and 0.1 ml menadione (1 mM). Sections were incubated at 37°C for 10 min, post-treated in 4 per cent formaldehyde and mounted in glycerine jelly.

Control sections for both NADH and NADPH dehydrogenases were incubated in the presence of dicumarol, a selective inhibitor for diaphorases.

RESULTS

Macroscopic Observations

No mortality or clinical signs of lead intoxication were obsereved for any individual of the dose groups over the entire period of the study. Kidney weight was slightly higher in lead exposed rats and kidneys from all lead treated rats showed swelling and an increase in the ratio of kidney/body weight in comparison to kidneys of the control rats.

No change in the liver weight was recorded due to lead treatment.

Histological Alterations

In comparison with respective control rats, lead had has produced distinct progressive tubular and glomerular alterations. Tubular changes occurred earlier than those of the glomerular ones and included anisokaryosis, nuclear pyknosis, karyomeglay, development of intranuclear and cytoplasmic inclusions together with tubular dilation, necrosis, vacuolization and tubular hyperplasia. The glomerular alterations were mainly mesangial hypercellularity and segmental proliferation. Nuclear alterations were seen in all lead-treated rats form one month and onword. In the beginning of lead exposure, the inclusion bodies were small but their frequency and sizes increased with duration of exposure to lead up to 8 months. Tubular damages also included degeneration, swelling, dilation, vacoulization, thickening of the tubular basement membranes and epithelial necrosis. These alterations appeared early with high doses of lead acetate trihydrate at 2% for 4 months and more of treatment. Focal tubular damage was seen at 0.5% for 5 months of exposure while isolated tubular damage was observed at 2% for 3 months and more of exposure. Tubular atrophy first appeared at 5 months with some increased in severity thereafter.

Focal cellular necrosis was observed in some of the renal proximal tubules of lead-treated rats. The necrotic tubules showed occasional absence of the proximal tubular brush border at 2% of lead acetate trihydrate. Vacuolar degeneration was observed mainly in the distal segment of the proximal tubules, while tubular vacuolization was seen in all treated rats up to 3 months of lead exposure but became rare thereafter. Alterations in the glomeruli of lead-treated rats started to appear in rats received 1% lead acetate trihydrate or more for seven months or more in the form of mesangial hypercellularity, sclerotic changes and segmental mesangial proliferation. These alterations were increased with increasing the lead doses and period of exposure and became mild at the 8th month of treatment. In addition, mesangial proliferation became more obvious in the kidneys of lead-treated rats in comparison with the control ones. Also, the glomerular basement membranes of some glomeruli became thickened and stumpy blocked in the kidney of rats received 2% of lead acetate trihydrate for 7 months and more.

The distribution patterns, sites of activities, intensity of reactions and histochemical characterization of the investigated renal dehydrogenases in the control and treated rats were as follows:

Succinate Dehydrogenase

In the control kidneys, strong activity of SDH was demonstrated in the proximal convoluted tubules and the macula densa. A faint activity was detected

Fig. 1a. Light Micrograph of the SDH Activity of Normal Rat Kidney

A strong activity is seen in the proximal convoluted tubules while no activity is observed in the glomeruli. × 50

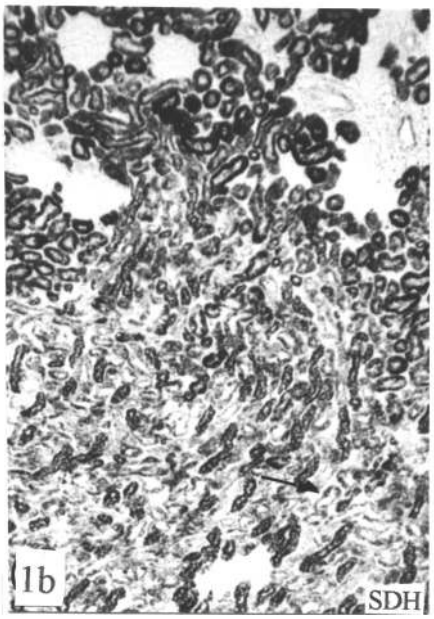

Fig. 1b. Light Micrograph of the SDH Activity in the Kidney of Lead Treated Rat Received 4% Lead Acetate in the Drinking Water for 7 Months

SDH reaction is decreased in the inner edge of the cortex. × 50

in the distal tubules, the juxtaglomerular cells and the ascending thick limb of the loop of Henle, while no activity was observed in the glomeruli (Fig. 1a) and the medulla.

In the treated kidneys, a considerable reduction in SDH activity was observed mainly in the pars recta in the inner edge of the cortex of rats received 1 per cent lead acetate trihydrate or more for continuous 6 months and more (Fig. 1b). The activity of this enzyme was not affected in pars convoluta of the proximal tubules.

The enzyme activity in the kidneys of rats received lower doses less than 1 per cent lead acetate trihydrate showed the same pattern of SDH activity in the control kidneys.

α-Glycerophosphate Dehydrogenase

A strong reaction of αGPDH was demonstrated in the pars recta, thick ascending limb of Henle's loop and macula densa. The activity of this enzyme was moderately demonstrated in the pars convoluta, distal collecting tubules and in the collecting ducts in the cortex and the outer zone of the medulla as well as in the medullary interstitial cells. The reaction was weak in the glomeruli, distal convoluted tubules, Goormaghtigh and juxtaglomerular cells. No activity was demonstrated in the thin limb of Henle's loop and the collecting ducts of the papillary inner medulla. (Fig. 2a).

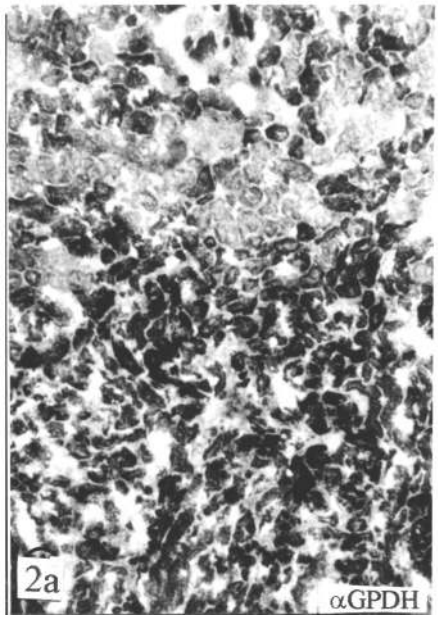

Fig. 2a. Light Micrograph of the αGPDH Activity of Normal Rat Kidney

A prominent reaction is seen in the pars recta. No αGPDH stain was seen in the thin limb of Henle's loop. × 80

In the treated kidneys, a significant increase in the activity of αGPDH was observed in the glomeruli and the collecting tubules of rats received 2 per cent lead acetate trihydrate and more for continuous 5 months and more (Fig. 2b). The αGPDH activity increased with increasing the lead doses and period

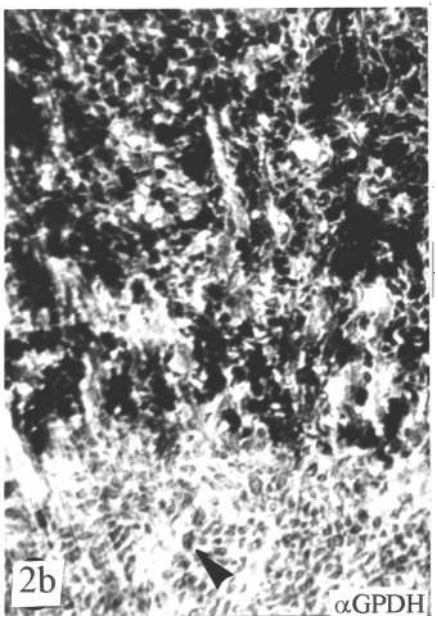

Fig. 2b. Light Micrograph of the αGPDH Activity in the Kidney of Lead Treated Rat Received 2% Lead Acetate in the Drinking Water for 7 Months

The activity of αGPDH reaction is increased in the glomeruli and appeared in the inner stripe of the outer medulla. × 80

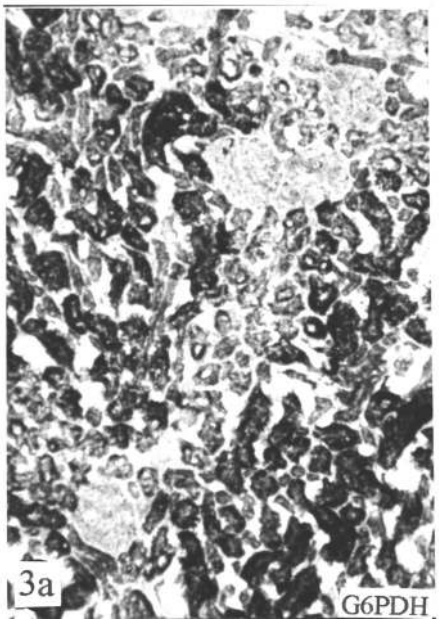

Fig. 3a. Light Micrograph of the G6PDH Activity of Normal Rat Kidney

The reaction is seen in the pars recta but faint activity is observed in the distal tubule. × 80

of exposure. A considrable increase in the activity of this enzyme was also noticed in the medullary interstitial cells at 4 per cent lead acetate trihydrate at the same length of exposure, while the activity in pars recta was not affected.

Glucose-6-Phosphate Dehydrogenase

In control kidneys, the activity of G6PDH was localized in the cytoplasm of the renal cells. High activity of this enzyme was seen in the macula densa and the pars recta while lesser activity was detected in the proximal convoluted tubules especially in the cortical portion (Fig. 3a). The activity was faint in the distal tubules and the pars convoluta of the proximal convoluted tubules but absent in the Goormaghtigh and the juxtaglomerular cells. A faint reaction for this enzyme was also observed in the mesangial cells.

A marked increase in the activity of G6PDH was seen in the kidneys of lead treated rats. The increase was mainly in the pars recta and to lesser extent in the ascending thick segment of the loop of Henle (Fig. 3b). The alteration in the activity of G6PDH appeared early after 2 months of continuous treatment at 0.5 per cent and more of lead acetate trihydrate and increased in activity thereafter with increasing the lead doses and period of exposure.

The enzyme activity in the kidneys of rats received less than 0.5 per cent lead acetate trihydrate showed the same pattern of G6PDH in the control kidneys.

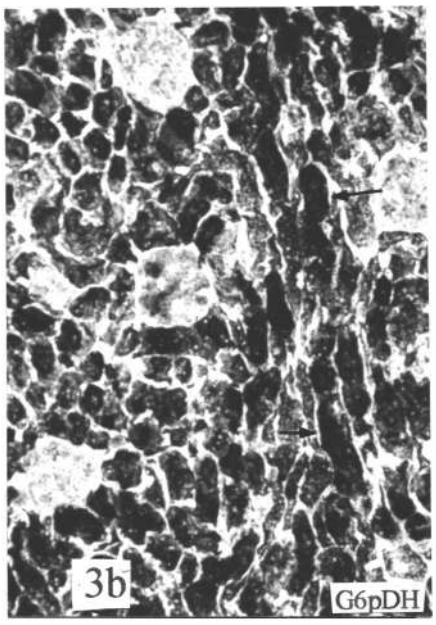

Fig. 3b. Light Micrograph of the G6PDH Activity in the Kidney of Lead Treated Rat Received 1% Lead Acetate in the Drinking Water for 7 Months

The reaction is increased in the pars recta and the ascending thick segment of the loop of the Henle. × 80

Fig. 4a. Light Micrograph of the LDH Activity of Normal Rat Kidney

A strong activity is seen in the cortical segments of the renal tubule but no reaction is observed in the medulla. × 80

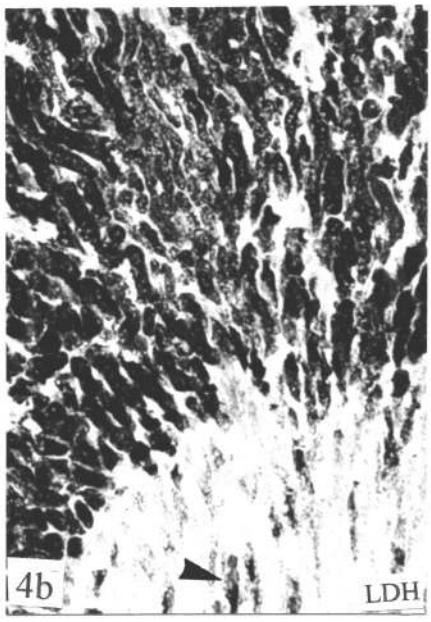

Fig. 4b. Light Micrograph of the LDH Activity in the Kidney of Lead Treated Rat Received 2% Lead Acetate in the Drinking Water for 5 Months

LDH reaction is appeared in the outer stripe of the inner medulla and the interstitial cells. × 100

Lactate Dehydrogenase

The kidneys of the control rats showed a strong reaction of LDH in the macula densa and pars recta of the renal tubule. A considerable activity was detected in the epithelial lining of the pars convoluta of distal convoluted tubules and in the cells of the collecting ducts. A faint stain appeared in the glomeruli, Goormaghtigh and juxtaglomerular cell while no staining was seen in the inner medullary zone (Fig. 4a). The activity of LDH was also seen in the thick ascending limb of of Henle's loop in the outer stripe of the medulla.

In the kidneys of lead treated rats, LDH activity increased in the different segments of the nephron in the cortex and the medulla especially in renal tubule near the renal cortex and to a lesser extent in the ascending segment of the loop of Henle (Fig. 4b). The continuous treatment of lead acetate trihydrate at 2 per cent and more for 5 months and more showed a considerable reaction in the interstitial cells of the medulla.

Malate Dehydrogenase

The activity of this enzyme in the kidneys of control animals was strongly demonstrated in the pars recta and to lesser extent in the distal convoluted tubules and ascending loop of Henle (Fig. 5a). A faint reaction was seen in the macula densa and juxtaglomerular cells.

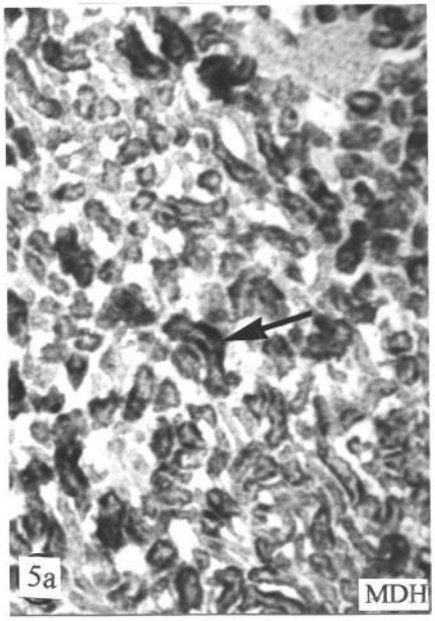

Fig. 5a. Light Micrograph of the MDH Activity of Normal Rat Kidney

A considerable activity is seen in the pars recta and the distal portions of the renal tubules while no staining is present in the glomeruli. × 80

The kidneys of the treated rats showed marked reduction in the activity of MDH in the straight portion of the renal tubule descends within the cortex and medulla that appeared after 4 months of treatment with 1 per cent of lead acetate trihydrate with

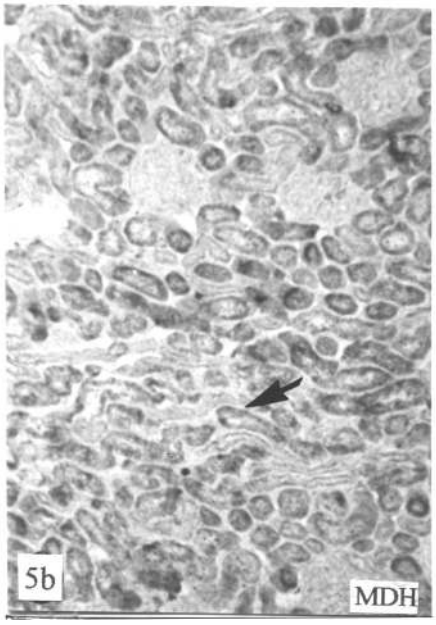

Fig. 5b. Light Micrograph of the MDH Activity in the Kidney of Treated Rat with Lead Acetate at 4% for Continuous 7 Months

A marked reduction of MDH activity is seen in different segments of the renal tubule. × 80

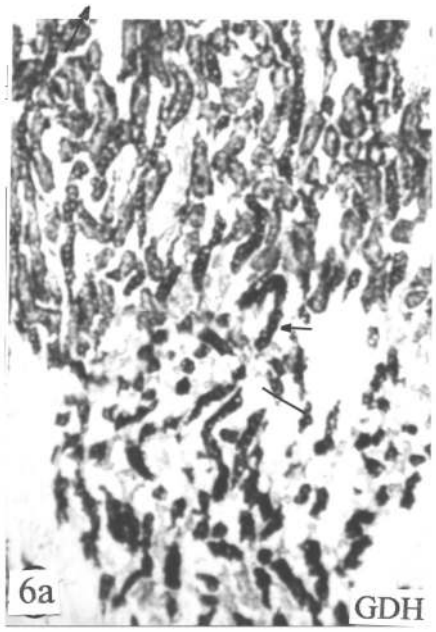

Fig. 6a. Light Micrograph of the GDH Activity of Normal Rat Kidney

The stain is prominent in the pars recta and the ascending thick segment of the loop of Henle. × 80

more reduction in the acxtivity of this enzyme thereafter. A marked reduction in the activity of this enzyme was clear in all parts of the renal tubule (Fig. 5b) and the juxtaglomerular apparatus at all levels of lead after 7 months of exposure.

Glutamate Dehydrogenase

The kidneys of the control rats showed strong activity of GDH in the epithelial lining of the pars recta and the ascending thick segment of the loop of Henle (Fig. 6a). A moderate reaction was detected in the cells lining the collecting tubules and to a lesser extent in the intraglomerular mesangial cells while the reaction was faint in the interstitial medullary cells.

A distinct reduction of glutamate dehydrogenase activity was demonstrated in the kidneys of rats treated at the level of 1 per cent lead acetate trihydrate and more for 4 months and more. The reduction in the activity of this enzyme was more prominent in the medulla than the cortical zone especially in the straight portion of the renal tubule and in the collecting ducts within the medulla. A considerable change was observed in the activity of GDH in the ascending thick segment of the loop of Henle (Fig. 6b).

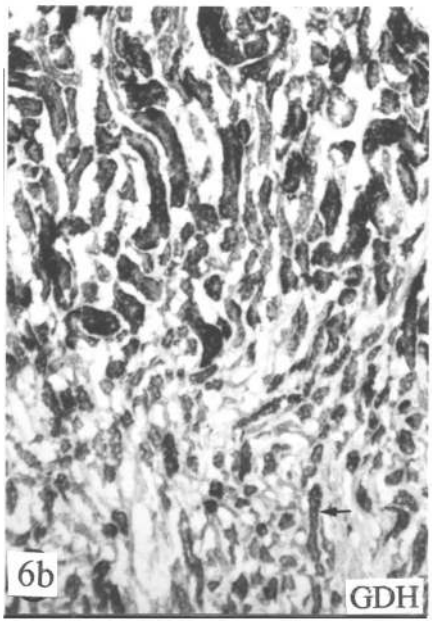

Fig. 6b. Light Micrograph of the GDH Activity in the Kidney of Lead Treated Rat Received 4% Lead Acetate in the Drinking Water for 3 Month

The reaction is decreased in the straight portion of the renal tubule. × 80

Isocitrate Dehydrogenase

In control rats, the activity of this enzyme was strong in the epithelial lining of the distal convoluted tubules and to a lesser extent in the macula

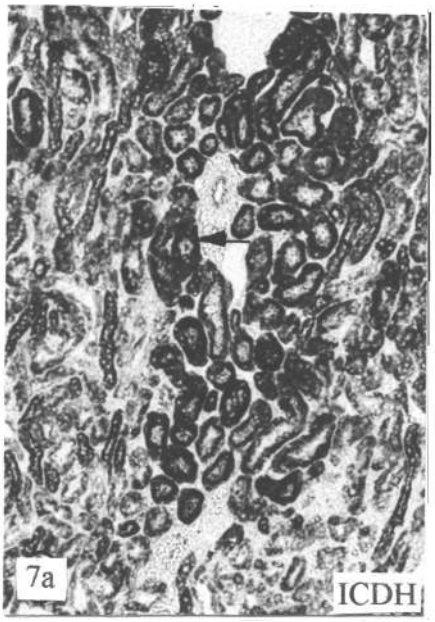

Fig. 7a. Light Micrograph of the ICDH Activity of Normal Rat Kidney

The reaction is strong in the macula densa and the distal convoluted tubules is seen. × 80

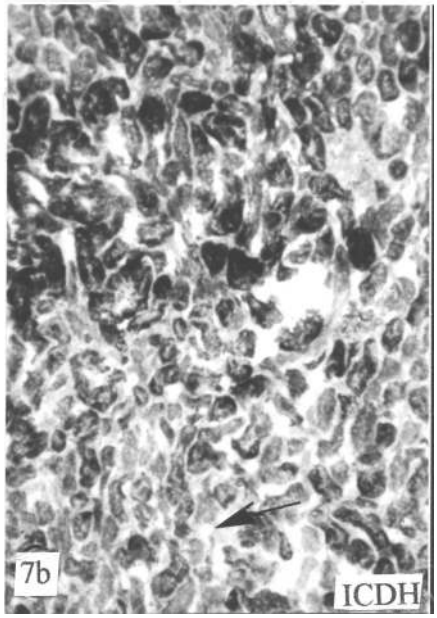

Fig. 7b. Light Micrograph of the ICDH Activity in the Kidney of Lead Treated Rat Received 2% Lead Acetate in the Drinking Water for 5 Months

A considerable reduction in the cortico-medullary junction is seen. × 80

densa while no activity was detected in the Goormaghtigh cells (Fig. 7a). A considerable activity of isocitrate dehydrogenase was also seen in the beginning of the pars convoluta and to a lesser extent in posterior and the cortical straight portions of the proximal convoluted tubule.

The kidneys of the lead treated rats showed a considerable decrease in ICDH activity in the cortical and medullary portions of the renal tubule (Fig. 7b). The alteration in the activity of this enzyme appeared at 2 per cent lead acetate trihydrate and more for 5 months and more of continuous treatment with more reduction in the activity of this enzyme thereafter.

NADH Dehydrogenase

A prominent activity of this enzyme was noticed in the distal convoluted tubules and to a lesser extent in the macula densa (Fig. 8a). A considerable reaction was observed in the straight portions of the renal tubules while Goormaghtigh cells showed weak activity. Almost no activity was demonstrated in the juxtaglomerular cells, thin loop of Henle and collecting ducts.

The activity of NADH dehydrogenase was greatly increased compared with the control animals. The increase in the activity of this enzyme was mainly seen in the pars recta and the thick loop of Henle and became visible in the collecting tubules

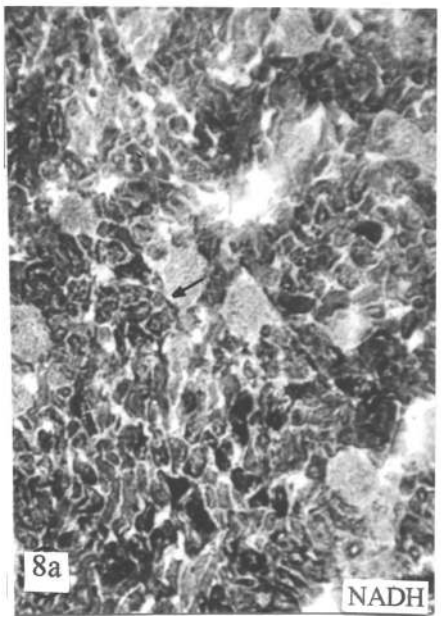

Fig. 8a. Light Micrograph of the NADH Dehydrogenase Activity of Normal Rat Kidney

A considerable reaction is observed in the epithelial lining of the distal portion of the renal tubules while no reaction is observed in the glomeruli. × 80

(Fig. 8b). The change was prominent in the rats receiving lead acetate trihydrate at 2 per cent or more for continuous 5 months and more, where most activity was seen therafter with increasing the lead dose.

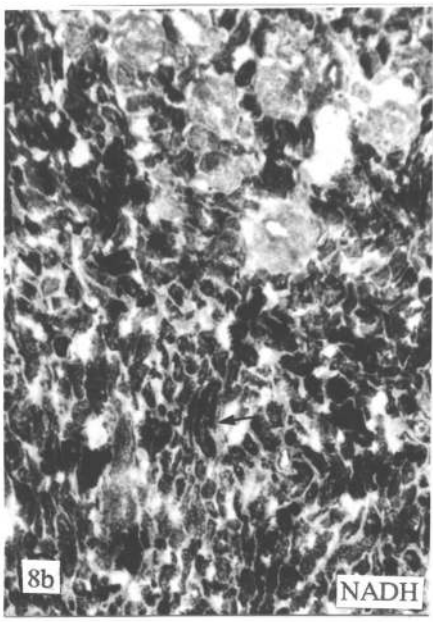

Fig. 8b. Light Micrograph of the NADH Dehydrogenase Activity in the Kidney of the Lead Treated Rat with 2% Lead Acetate for 5 Months

Compared with Fig. 8a, the reaction in the renal tubules is increased. × 80

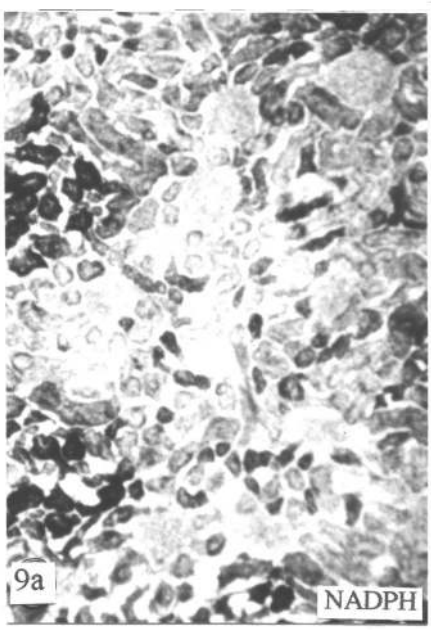

Fig. 9a. Light Micrograph of the NADPH Dehydrogenase Activity of Normal Rat Kidney

× 80

NADPH Dehydrogenase

In control rats, a strong activity of NADPH dehydrogenase was seen in the beginning of pars convoluta and to a lesser extent in the macula densa, distal convoluted tubules and the cortical portion of pars recta (Fig. 9a). A weak reaction was noticed in the juxtaglomerular cells and the posterior portion of the pars recta. No activity of this enzyme was demonstrated in the Goormaghtigh cells.

The kidneys of treated rats showed considerable increase in the activity of this enzyme in the epithelial lining of pars convoluta (Fig. 9b) with lead acetate trihydrate at 4 per cent or more for continuous 6 months and more, while no change in the activity was obseved in the other portions of the renal tubule and the juxtaglomerular apparatus. The enzyme activity in the kidneys of rats received lower doses less than 4 per cent lead acetate trihydrate showed the same pattern of reaction in the control kidneys.

There was high degree of concordance between the findings of each two rats kidneys at each time point and % lead ingestion for all tested dehydrogenases except one kidney showed unilateral cysts seen after 8 months of 4% lead treatment and was excluded from the evaluation of dehydrogenases activities.

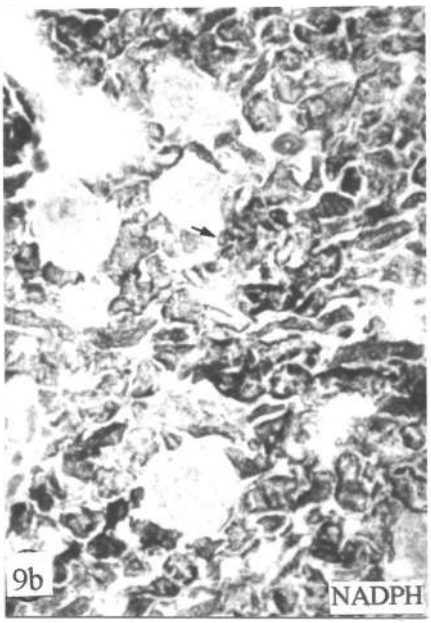

Fig. 9b. Light Micrograph of the NADPH Dehydrogenase Activity in the Kidney of the Lead Treated Rat with 4% Lead Acetate Trihydrate for 8 Months

A considerable increase in the activity of this enzyme is seen in the pars convoluta. × 80

DISCUSSION

The results of the present investigation have shown a considerable decrease in the activity of succinate-, malate-, glutamate-, and isocitrate dehydrogenase. Succinate dehydrogenase possesses SH

groups on which the enzymatic activity depends and lead is know to interfere with these groups.[32] Succinate dehydrogenase participates in the aerobic oxidation of carbohydrates in the citric acid cycle and is bounded to the inner mitochondrial membrane. The reduction of succinate dehydrogenase activity due to lead intoxication might indicate injured mitochondrial functions and reduction in carbohydrates oxidation. Our results are in agreement with the biochemical findings of Nehru and Kaushal,[6] where SDH was significantly inhibited following lead poisoning but in dispute with the findings of some biochemical studies where lead exposure had no influence on succinate dehydrogenase activity in the liver and kidney homogenates of rats fed for 6 months with 1 per cent lead acetate diet.[16,33,34] Similar results were obtained in the heart tissue.[35]

The reduction in the activity of malate dehydrogenase due to lead intoxication seen in the present histochemical results is in agreement with the results of biochemical investigation on the activity of this enzyme in the kidney homongenate, and muscle fibres of intoxicated rats.[33,36] Malate dehydrogenase converts malate to oxaloacetate, the final reaction in the Krebs cycle.

The decrease in the activity of glutamate dehydrogenase in the present study is in agreement with the results of biochemical studies carried on the activity of this enzyme in kidney tissue of lead poisoned rats, rabbits and guinea pigs.[33,37]

NAD-dependent isocitrate dehydrogenase is structuraly bounded to the mitochondria and participates in the Krebs cycle. It has been reported that the activity of this enzyme was increased in the muscle fibers of lead intoxicated rats.[36]

The results of the present investigation have shown an increase in the activities of glucose-6-phosphate-, lactate-, α-glycerophosphate-, NADH- and NADPH dehydrogenase. Glucose-6-phosphate dehydrogenase catalyses the first step of oxidation in the hexose monophosphate pathway by which glucose may enter the pentose monophosphate shunt and producing NADPH which is required as hydrogen donor for reactions of various biochemical pathways. This enzyme plays an important role in the regulation of sugar metabolism and determines whether glucose shall undrergo glycolysis or be utilized via the pentose phosphate pathway. The increase in the activity of glucose-6-phosphate dehydrogenase due to lead intoxication might indicate an increased demand to generate reducing power in the form of NADPH under the oxidative stress fail induced by lead. Lactate dehydrogenase playes an important role in the intermediary metabolism as a link between amino acid metabolism and the citric acid cycle where it converts lactate into pyruvate. The increase in the activity of this enzyme due to lead intoxication in the present study is in agreement with the biochemical findings of Secchi[37] but in contradiction with the findings of Iannaccone *et al.*,[33] where the activity of lactate dehydrogenase was unmodified in the kidney homogenate of rats fed for 6 months with 0.1 per cent lead acetate diet and of Yagminas *et al.*,[38] where the activity of this enzyme was lowered in the kidney of lead acetate treated rats. The varieties in the activity of renal dehydrogenases due to lead intoxication obtained by different investigators could be due to the variations in the experimental conditions such as the level of exposure, duration, route of administration and animal species used in the experiment.

The reaction product of α-glycerophosphate dehydrogenase, glycerol-3-phosphate, is a key substance for the synthesis of triglycerides and phospholipids. The increase in the activity of this enzyme due to lead intoxication might reflect a need for more NAD-NADH shuttle between the cytoplasm and mitochondria.

NADH and NADPH dehydrogenase activities were increased in the kidneys of lead treated rats of the present study. These enzymes are considered a bybass of the phosphorylating electro-transfer pathway between NADH and NADPH and cytochrome b.[39] The increase in the activity of these enzymes due to lead intoxication might be a need to increase the capacity of intracellular oxidation where coenzymes are generated into their oxidized state.

A considerable change in the activity of renal dehydrogenases was observed in the cells of the macula densa and the juxtaglomerular apparatus. This might indicate that lead poisoning affects filtrate rate which is monitered by these cells. The increase in the activity of dehydrogenases in the intraglomerular mesangial cells may reflect a phgacocytic activity due to lead intoxication.

Most of the dehydrogenases investigated in the present histochemical study are structurally bounded in the matrix and the intact inner mitochondrial membrane. The findings led us to suggest that the alterations in the activity of renal dehydrogenases might be due to deleterious actions exerted by lead on the cytoplasmic organelles especially the mitochondria. The inhibition of succinate-, malate- and isocitrate dehydrogenase which are mitochondrial enzymes

and involved in the citric acid cycle might support this suggestion and indicate a reduction in the aerobic metabolic processes especially the electron transport and oxidative phosphorylation of the renal cells due to lead intoxication. In addition, these histochemical alterations let us to conclude that the changes in the activity of dehydrogenases due to lead intoxication might represent responses to the need for an adaptation to the catabolism of the degrading damaged renal cells structures or to partial impairments of their functions.

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