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Original Article

A Histological Study on the Effect of Lead Acetate on the Developing Metanephros in Rabbit

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ABSTRACT

Aim of the Work: This study was carried out to show the effect of lead acetate on the developing metanephros in rabbit.

Material and Methods: A total number of 60 rabbit embryos and newborn rabbits were divided into 2 groups, control (n=30) and experimental (n=30). The pregnant mothers of the experimental group received 15 mg / kg BW lead acetate by intragastric intubation on the 10th day of pregnancy until birth. The control group received sodium acetate on the same regimen. For light microscopic study, the specimens were processed on gestational days 16, 20, and 24 and the age of newborn and stained with haematoxylin and eosin and periodic acid Schiff reagent. In two age groups, 24 days prenatal and newborn (control and treated), morphometric measurements were performed to study the diameter and the number of glomeruli and tubules. Also, the glomerular space was measured. Kidney specimens from fetuses on gestational day 26 and from newborns were processed for transmission and scanning electron microscopic study.

Results: The control animals showed that the metanephros appeared as a circumscribed mass behind the caudal part of the mesonephros at the age of 16 days prenatal. At the age of 20 days prenatal beneath the renal capsule, the metanephros showed a developed nephrogenic zone containing different stages of nephron development. In the succeeding ages, this nephrogenic zone increased in thickness until birth where it was still persistent. In the experimental group, there were dispersion and disorganization of the metanephric cells and the nephrogenic zone at early ages. In the succeeding ages, cavitations, dilated vascular spaces, increased interstitial tissues with degenerative changes in the glomeruli and tubules of the kidney were observed. At 26-day-old embryo, semithin sections showed degeneration of the cells of the proximal convoluted tubules with irregular vacuolations, clumped intracytoplasmic dark granules and severely damaged brush borders. Cells of the distal convoluted tubules were degenerated. The glomeruli showed dilated congested capillaries with some glomeruli showing dilated glomerular space. These manifestations became more apparent in leaded newborns. Ultrathin sections showed injured epithelial cells of the proximal convoluted tubules with damaged microvilli, vacuolated cytoplasm, swollen mitochondria and degeneration of their cristae. Inclusion bodies and metal deposits were observed in the cytoplasm. In leaded newborns, massive degeneration was observed. Scanning electron microscopy showed marked degeneration of the glomeruli and tubules as compared with the control. Morphometric measurements showed significant reduction in the glomerular diameter, the tubular diameter and the number of glomeruli and tubules per mm with significantly widened glomerular space in lead- treated animals in comparison with the control animals.

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Key Words: Lead acetate, kidneys, metanephros, morphology, rabbit, light microscopy, scanning electron microscopy.

INTRODUCTION

Environmental lead toxicity is an old but persistent public health problem throughout the world and children are more susceptible to lead than adults *(Ahamed and Siddiqui, 2007)*. The general population is exposed to lead from air and food in roughly equal proportions *(Järup, 2003)*. Lead freely crosses the placenta *(Papanikolaou, et al. 2005)*. Consequently, gestational lead poisoning is not only harmful to the mother but also to the developing fetus, invariably producing congenital lead poisoning *(Shannon, 2003)*. In humans, lead intake can results in a wide range of biological effects depending upon the level and duration of exposure. Effects may range from inhibition of en-

zymes to the production of marked morphological changes and death *(Massanyi, et al. 2007)*. The lead cellular toxicity depends upon the metal's interaction with biochemical systems that regulate many cell functions *(Errede, et al. 2001)*.

Chronic kidney disease represents a major global public health concern. Lead nephropathy, characterized by chronic tubulo-interstitial nephritis, is a well-known risk of chronic, high-level lead exposure *(Ekong, et al. 2006)*. Moreover, low socioeconomic status is a risk factor for both lead exposure and diseases that increase susceptibility. Investigations carried out on the lead-exposed workers as well as animals by different experimental models have clearly demonstrated the cytotoxicity of this metal and have also suggested a lead carcinogenic effect *(Goyer, 1990; Landrigan, et al. 2000)*. Cases of chronic renal failure have been reported in adults who ingested large amounts of leaded paint during childhood, and in workers with a long history of occupational lead exposure *(Emmerson, 1973; Bennett, 1985)*.

Very few literatures have been found on the effect of lead on the developing metanephros. The aim of this study was to assess the potential renal side effects of the prenatal exposure to lead in an animal model that is very close to the human situation.

MATERIAL AND METHODS

In this work, a total number of 60 rabbit embryos and newborn were used. The animals were divided into 2 groups, control (n=30) and experimental (n=30). The pregnant mothers were housed in cages under standardized animal house conditions. Pregnant mothers of the experimental group received lead acetate dissolved in distilled water on the 10^{th} day of pregnancy and it was repeated daily in a dose of 15 mg / kg body weight by intragastric intubation until birth. The control group received sodium acetate on the same regimen.

For light microscopic study, fetuses were removed from pregnant mothers under ether anaesthesia on gestational days 16, 20, and 24 and the age of newborn (6 control animals and 6 treated animals in each age group). Specimens were fixed in Bouin's fluid, dehydrated in ascending grades of alcohol, cleared, embedded in paraffin and serially sectioned at 10 µm. Sections were stained with haematoxylin and eosin stain and periodic acid Schiff reagent *(Drury and Wallington, 1980)*.

For electron microscopic study, kidney specimens from fetuses on gestational day 26 and from newborns (3 control animals and 3 treated animals in each age group) were fixed in 5% coccodylate buffered gluteraldehyde.

For examination under transmission electron microscopy, about 2 mm thick slices were processed and embedded in Araldite mixture. Semithin sections of one - micron thickness were cut with a glass knife in KLB ultramicrotome and stained with toluidine blue. Other ultrathin sections were cut, stained with uranyl acetate and lead citrate and examined with JEM - 100 CX11 electron microscope.

For scanning electron microscopy, blocks of kidney tissues were processed, subjected to CO^2 in a critical point drying apparatus and coated with a thin layer of gold in a vacuum evaporator. The blocks were examined and photographed.

Morphometric methods: In two age groups, 24 days prenatal and newborn (control and treated), the glomerular diameter, glomerular space and tubular diameter were measured using the scale slide. In addition, the number of glomeruli per mm and tubules per mm was counted in a linear area of defined length. The length of this line was measured using the scale slide. The proximal and distal convoluted tubules were only counted. Five slides from each animal were used for count and measurements. The slides were chosen from the mid area of the kidney and the hilum was taken as a guide for this site. The equation used was: Magnification = length in image/natural length

Statistical analysis of the data was done using the student t-test and the data expressed in means and standard deviations.

RESULTS

16-day- old rabbit embryos: In control animals, the metanephros appeared as a localized circumscribed mass behind the caudal part of the well-developed mesonephros. Cells of the metanephric blastema, present just beneath the renal capsule, were condensed as crescent bodies (metanephric caps) around the ampullary terminal ends (collec-

ting tubules) of the ureteric bud (Fig.1). In lead-treated animals, there was dispersion and disorganization of the cells of the metanephros. The cells of the metanephric cap were less differentiated from the surrounding mesenchymal cells (Fig. 2).

20-day- old rabbit embryos: In control animals, the metanephros beneath the renal capsule showed a developed nephrogenic zone. There were different stages of nephron development (Fig. 3). The primitive glomeruli were apparent in the cortex while the most mature golmeruli were juxtamedullay. In addition, some tubules were apparent.

In lead-treated animals, there was disorganized and less developed nephrogenic zone. There were cavitations, dilated vascular spaces with an increase in the interstitial areas. In addition, there were less differentiated glomeruli (Fig. 4).

24- day- old rabbit embryos: As compared with the previous ages, the control animals showed well-developed cortical nephrogenic zone with different stages of nephron development (Figs. 5, 7). The tubules were more developed (Fig. 9). In sections stained with periodic acid Schiff, the brush border of the proximal convoluted tubules, the basement membranes and the glomeruli were intensely stained (Fig. 11), while the distal convoluted tubules showed no reaction.

In lead-treated animals, the developing kidneys were shrunken with apparent decreased cortical thickness and less developed nephrogenic zone (Figs. 6, 8). The glomeruli were scanty and decreased in size with retracted and atrophic glomerular tuft and dilated glomerular space. The tubules were also scanty with an increase in the interstitial tissue in relation to the parynchyma (Figs. 8, 10). There were cavitations and dilated vascular spaces (Figs. 6, 12). In sections stained with periodic acid Schiff, some proximal convoluted tubules showed less staining or discontinuity of the brush border (Fig. 12).

26- day- old rabbit embryos: In control animals, semithin sections showed that the cells of the proximal convoluted tubules were formed of rounded vesicular nuclei, well stained cytoplasm with multiple rounded vacuoles, and well developed brush border (Fig. 17). The cells of the distal convoluted tubules were characterized by scanty cytoplasm, and prominent nuclei. The majority of renal corpuscles presented a very thin glomerular space with densely packed cells (podocytes) of the visceral layer of the Bowman's capsule closely lined up against the cells of the parietal layer (Fig. 20).In ultrathin sections, cells of the proximal convoluted tubules showed euchromatic basal nuclei, mitochondria, multiple vacuoles and ribosomes with well-developed microvilli(Fig. 23). Cells of distal convoluted tubules showed large nuclei with scanty organelles (Fig. 29).

In lead-treated animals, cells of the proximal convoluted tubules were degenerated (Fig. 18) with irregular vacuolations of the cytoplasm and clumped intracytoplasmic dark granules. The brush borders were severely damaged. Cells of the distal convoluted tubules were vacuolated with irregular shape of the nuclei (Figs. 18-21). The glomeruli showed dilated congested capillaries (Fig. 21). Ultrathin sections showed injured epithelial cells of the proximal convoluted tubules with damaged microvilli (Figs. 24, 25). The cytoplasm showed areas devoid of cell organelles with swollen mitochondria and degeneration of their cristae. Inclusion bodies and metal deposits were observed in the cytoplasm (Figs. 24-26). Cells of the distal convoluted tubules showed inclusion bodies in the nuclei with some of these bodies seen entering from the cytoplasm to the nucleus (Fig. 30). Areas devoid of cell organelles with swollen and degenerated mitochondria were observed.

Newborn rabbits: The control animals showed an increase of the kidney size with still persistent well-developed cortical nephrogenic zone (Fig. 13). The glomeruli and tubules were more developed and showed normal appearance (Fig. 13). Periodic acid Schiff stain showed intense reaction of the brush border of the proximal convoluted tubules, basement membrane and glomeruli (Fig. 15).

In lead-treated animals; there was less developed nephrogenic zone. The tubules were scanty, smaller in size and showed cystic changes (Fig. 14). The proximal convoluted tubules showed focal loss of the brush border and basement membrane (Fig. 16). There were scanty glomeruli with atrophic capillary tuft and an increase in the glomerular space. Cavitations, dilated vascular spaces, and an increase in the interstitial tissue were observed (Figs. 14, 22).

Semithin sections showed marked degeneration of the proximal convoluted tubules, the distal con-

voluted tubules and the collecting tubules (Fig. 19). The glomeruli showed marked degeneration with thickened parietal layer of the Bowman's capsule, shrinkage of capillary tuft and widened glomerular space (Fig. 22).

Ultrathin sections showed massive degeneration of the cells of the proximal convoluted tubules (Fig. 28) with disfigured mitochondria and loss of microvilli in comparison with normal (Fig. 27). Metal deposits were observed in the cytoplasm.

Scanning electron microscopy showed marked degeneration and shrinkage of the glomeruli (Fig. 32) and tubules (Fig. 34) as compared with the control (Figs. 31, 33).

Morphometric results: In lead-treated animals, there was a significant reduction in the number of glomeruli per mm and the glomerular diameter with significant widening of the glomerular space in comparison with the control animals (Table 1).

In lead-treated animals; there was a significant reduction in the number of tubules per mm with shrinkage in the tubular diameter in comparison with the control animals (Table 2).

Table 1: Morphological parameters of the glomeruli in 24-day-rabbit embryos and newborn rabbits (control and lead treated).

Group	Prenatal (24 days)			Newborn (30 days)		
Parameter	C n=6	L n=6	P	C n=6	L n=6	P
Glomeruli/ mm	19.4± 3.8	14.4± 4.6	0.005 **	22.9±5.4	14.3±5.6	0. 001 **
Glomerular diameter	62.0±2.8	48.5±4.9	<0.0001 ***	67.5±6.5	58.4±7.4	0.02 *
glomerular space	6.9±0.2	11.5±0.9	<0.0001 ***	7.3±0.3	13.8±2.1	<0.0001 ***

Table 2: Morphological parameters of the tubules in 24-day-rabbit embryos and newborn rabbits (control and lead treated).

Group	Prenatal (24 days)			Newborn (30 days)		
Parameter	C n=6	L n=6	P	C n=6	L n=6	P
Tubule/ mm	32.3±7.2	24.5±7.9	0.04 *	37.3±9.1	27.7±8.4	0.02 *
Tubular diameter	52.3±6.1	46.9±2.8	0.008 **	56.7±2.4	53.4±3.2	0.01 *

P value: * (significant), ** (very significant), *** (extremely significant).

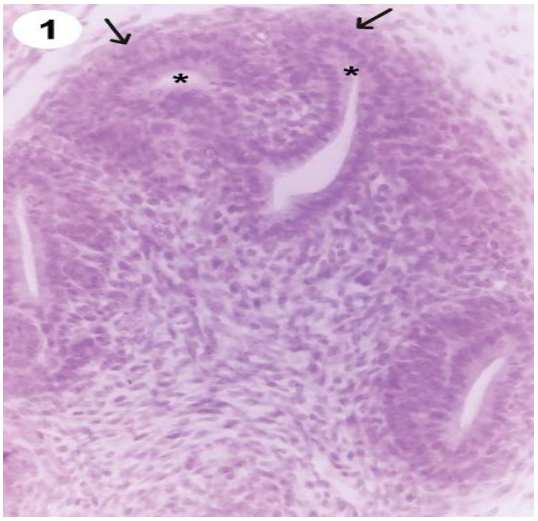

Fig. 1: A photomicrograph of metanephric kidney in 16-day-old control rabbit embryo showing metanephric caps (arrows) around the ampullary terminal ends of ureteric bud (*). Hx. & E.; X250

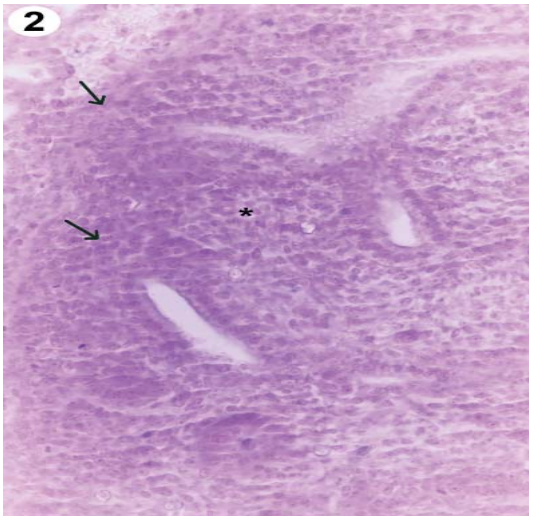

Fig. 2: A photomicrograph of metanephric kidney in 16-day-old lead treated rabbit embryo showing disorganization of the cells of the metanephros (*) and disorganized and less differentiated cells of the metanephric cap(arrows). Hx. & E.; X250

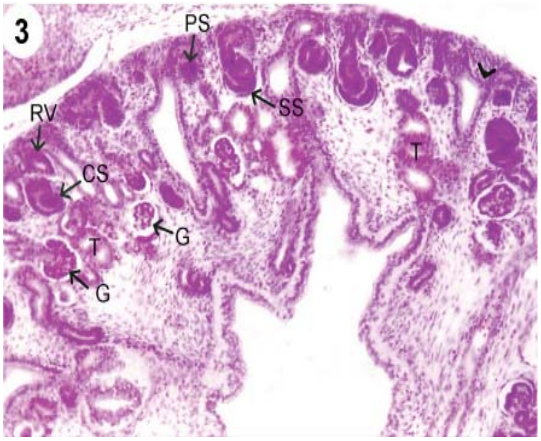

Fig. 3: A photomicrograph of the metanephric kidney in 20-day- old control rabbit embryo showing developed nephrogenic zone with different stages of nephron development. Note primitive sphere(PS), renal vesicle(RV), comma shaped stage(CS), S- shaped stage(SS), tubules(T), glomerulus(G) and the dichotomous division of ampullar extremity (arrowhead). Hx. & E.; X100

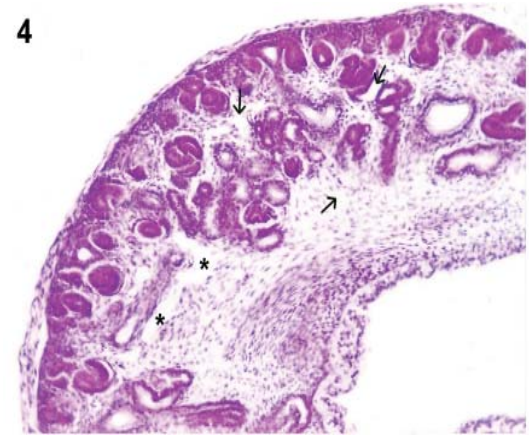

Fig. 4: A photomicrograph of the metanephric kidney in 20-day- old lead treated rabbit embryo showing disorganized and less developed nephrogenic zone and less differentiated glomeruli. Note cavitations (arrows) and dilated vascular spaces (*). Hx. & E.; X100

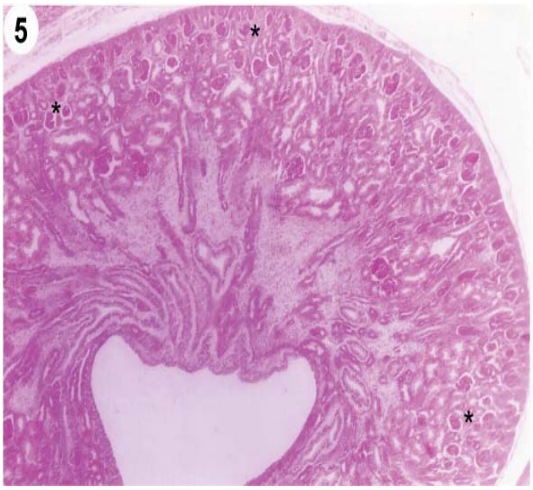

Fig. 5: A photomicrograph of the metanephric kidney in 24-day- old control rabbit embryo showing well developed nephrogenic zone (*) and more development of glomeruli and tubules. Hx. & E.; X40

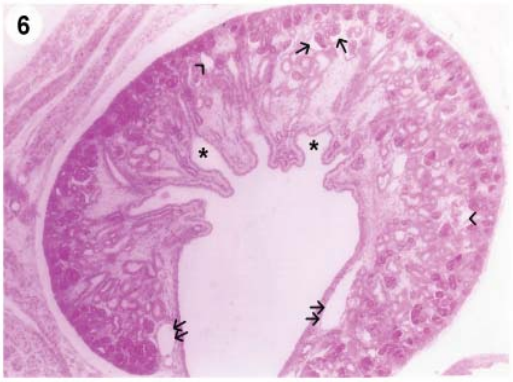

Fig. 6: A photomicrograph of the metanephric kidney in 24-day- old lead treated rabbit embryo showing less developed nephrogenic zone, less developed tubules, scanty and smaller glomeruli with retracted glomerular tuft and dilated glomerular space (arrows), cavitations (arrow heads), dilated vascular spaces (double arrows) and dilated calyces (*)and renal pelvis. Hx. & E.;X40

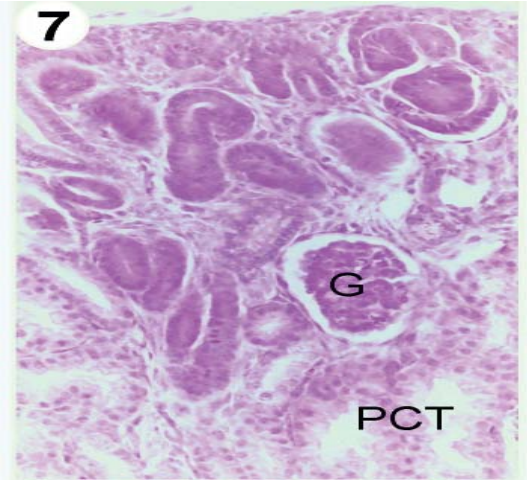

Fig. 7: A photomicrograph of the cortex of metanephric kidney in 24-day- old control rabbit embryo showing well developed nephrogenic zone with different stages of nephron development. Note glomerulus (G) and proximal convoluted tubules (PCT). Hx. & E.; X250

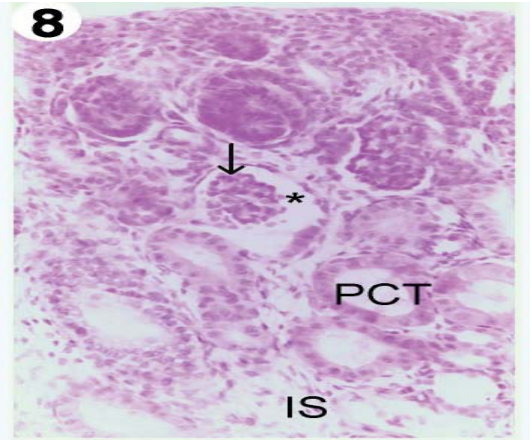

Fig. 8: A photomicrograph of the cortex of metanephric kidney in 24-day- old lead treated rabbit embryo showing less developed nephrogenic zone, less developed and dilated proximal convoluted tubules(PCT), scanty and smaller glomeruli with retracted glomerular tuft (arrow)and dilated glomerular space(*). Note the increase in the interstitial areas (IS). Hx. & E.; X250

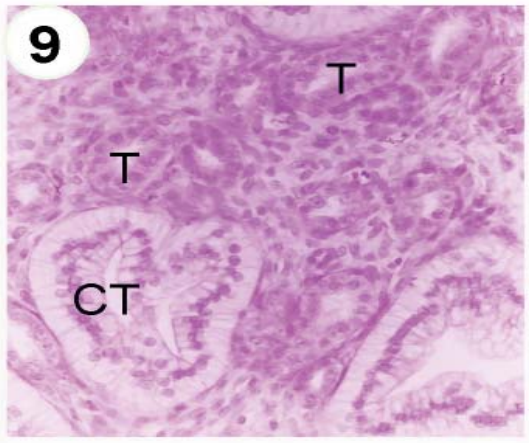

Fig. 9: A photomicrograph of the juxtamedullary region of the metanephric kidney in 24-day- old control rabbit embryo showing well-developed tubules (T). Note collecting tubules (CT). Hx. & E.; X250

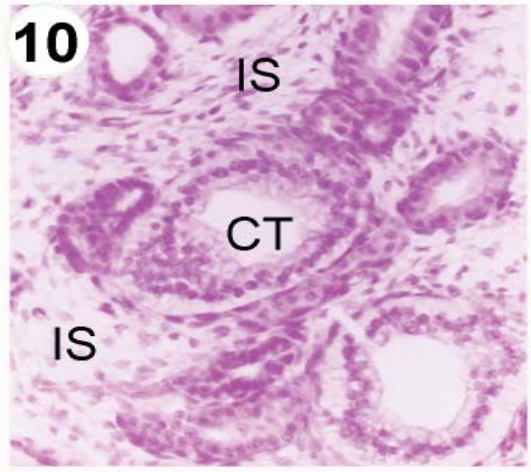

Fig. 10: A photomicrograph of juxtamedullary region of metanephric kidney in 24-day- old lead treated rabbit embryo showing an increase in the interstitial areas (IS). Note collecting tubules (CT). Hx. & E.; X250

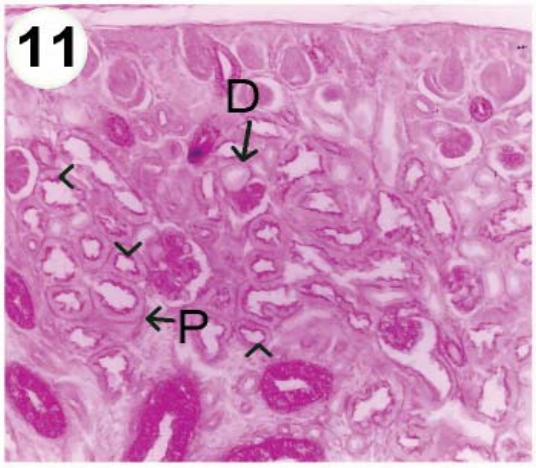

Fig. 11: A photomicrograph of the cortex of metanephric kidney in 24-day- old control rabbit embryo showing brush borders (arrow heads) of the proximal convoluted tubules (P). Note distal convoluted tubules (D). PAS; X100

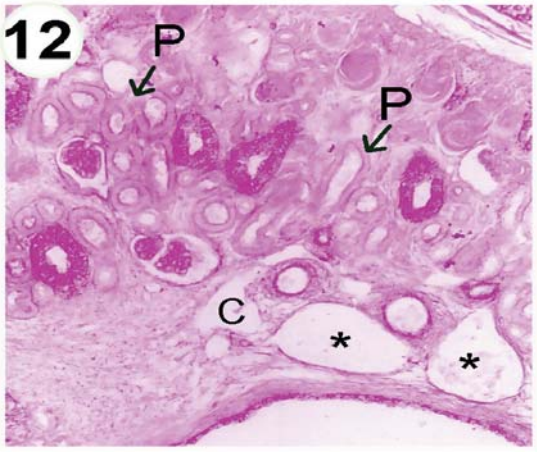

Fig. 12: A photomicrograph of the cortex of metanephric kidney in 24-day- old lead treated rabbit embryo showing less stained brush border of the proximal convoluted tubules (P), dilated vascular spaces (*) and cavitations(C). PAS; X100

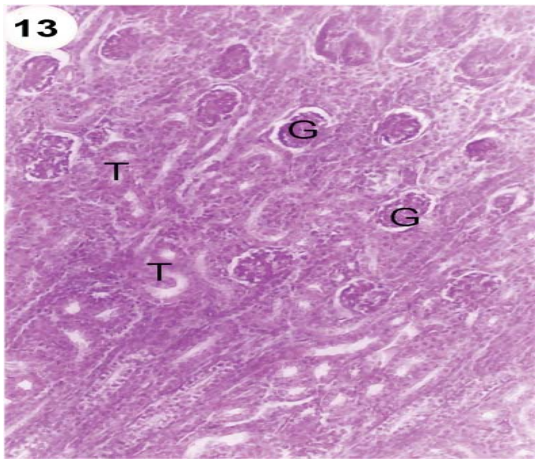

Fig. 13: A photomicrograph of the metanephric kidney in control newborn rabbit showing well-developed glomeruli (G) and tubules (T). Hx. & E.; X 100

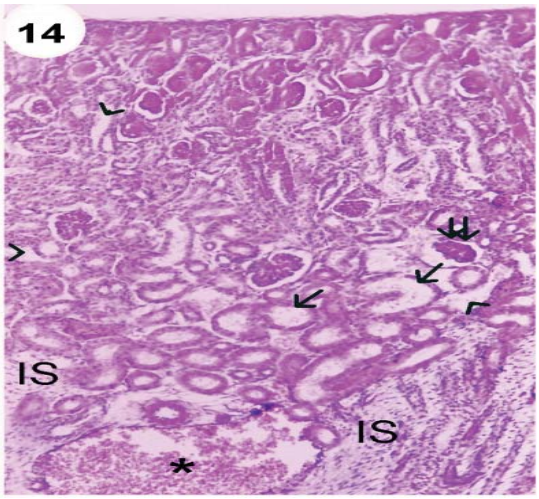

Fig. 14: A photomicrograph of the metanephric kidney in lead treated newborn rabbit showing marked disorganization of the nephrogenic zone, scanty atrophic glomeruli with an increase in the glomerular space (double arrows), dilated tubules (arrows), dilated vascular spaces (*), cavitations (arrow heads) and an increase in the interstitial areas (IS). Hx.&E.;X 100

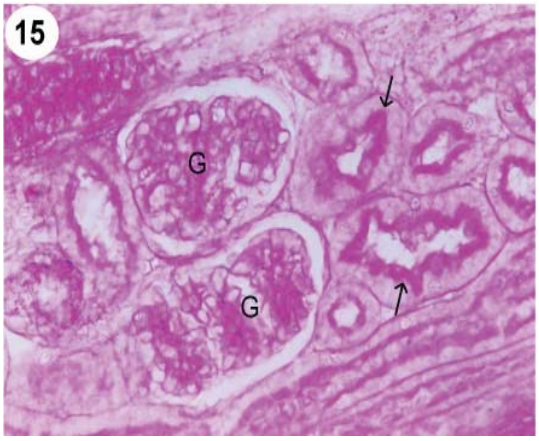

Fig. 15: A photomicrograph of the metanephric kidney in control newborn rabbit showing brush border of the proximal convoluted tubules (arrows). Note glomerulus (G) . PAS; X400

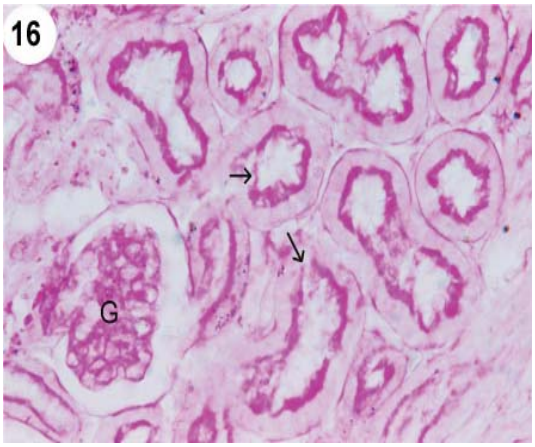

Fig. 16: A photomicrograph of the metanephric kidney in lead treated newborn rabbit showing less stained or disrupted brush border of the proximal convoluted tubules (arrows). Note glomerulus (G). PAS; X400

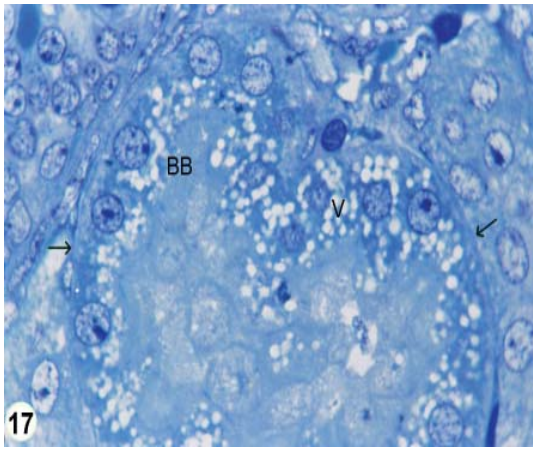

Fig. 17: A photomicrograph of semithin section of a proximal convoluted tubule in 26-day-old control rabbit embryo showing normal appearance of the cells. Note brush border (BB), rounded vacuoles (V) and basement membrane (arrows). Toluidine blue; X1000

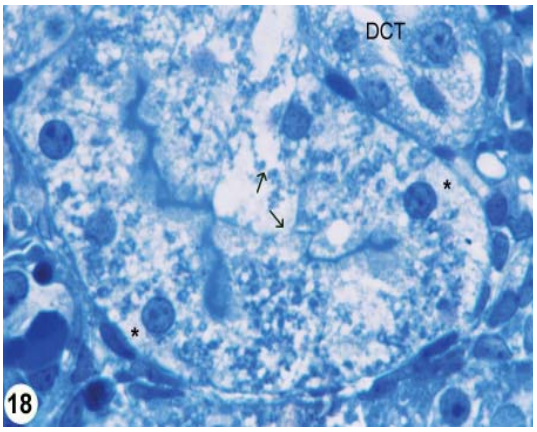

Fig. 18: A photomicrograph of semithin section of degenerated proximal convoluted tubule in 26-day-old lead treated rabbit embryo showing irregularly vacuolated cytoplasm (*) with loss of brush border (arrows). Note degenerated distal convoluted tubules (DCT). Toluidine blue; X1000

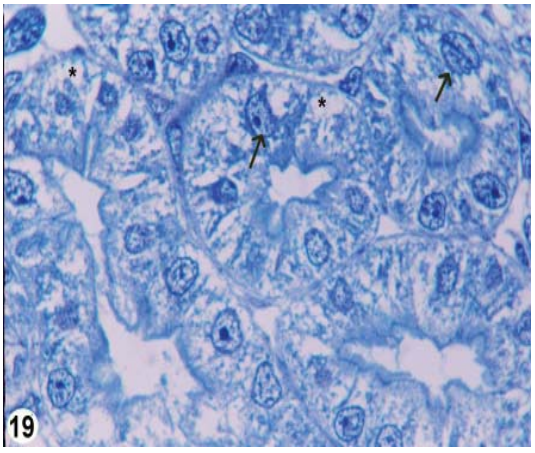

Fig. 19: A photomicrograph of semithin section of degenerated proximal convoluted tubules in lead treated newborn rabbit showing loss of brush border and irregularly vacuolated cytoplasm (*) with irregular shape of the nuclei (arrows). Toluidine blue; X1000

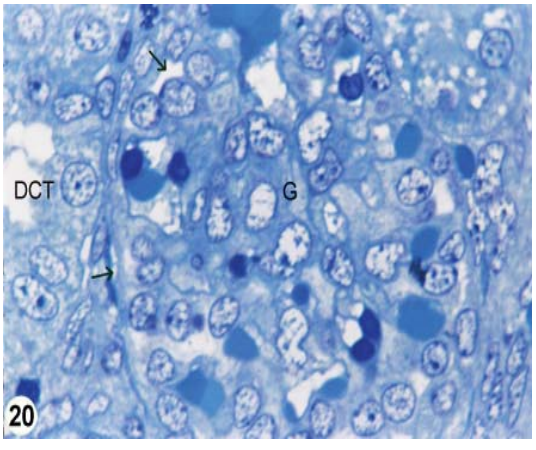

Fig. 20: A photomicrograph of semithin section of the metanephric kidney in 26-day-old control rabbit embryo showing well developed glomerulus (G) with a very thin glomerular space (arrows). Note distal convoluted tubules (D CT). Toluidine blue; X1000

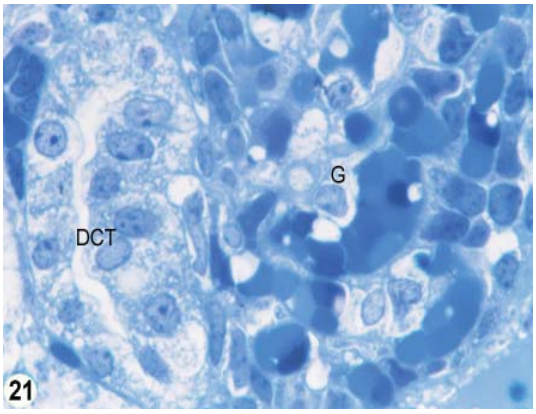

Fig. 21: A photomicrograph of semithin section of the metanephric kidney in 26-day-old lead treated rabbit embryo showing congested glomerulus (G).Note degenerated cells of the distal convoluted tubules (DCT). Toluidine blue; X1000

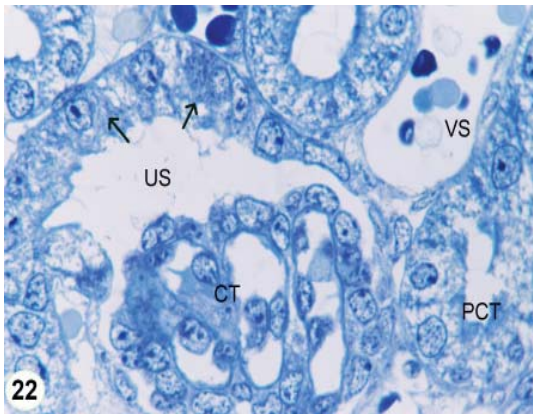

Fig. 22: A photomicrograph of semithin section of metanephric kidney in lead treated newborn rabbit showing glomerulus with thickened parietal layer of the Bowman's capsule (arrows), shrinkage of capillary tuft (CT) and widened glomerular space (US). Note dilated vascular space (VS) and degenerated proximal convoluted tubule (PCT). Toluidine blue; X1000

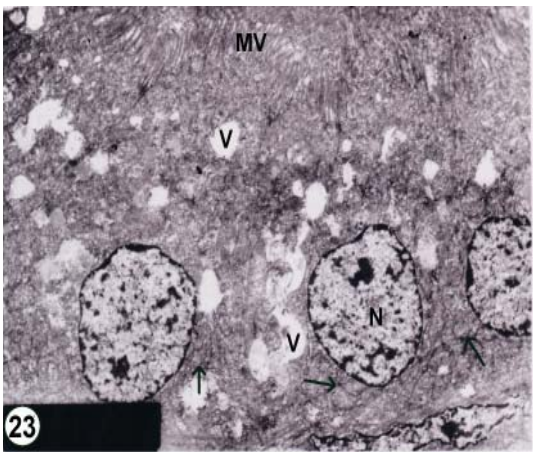

Fig. 23: An electron micrograph of cells of the proximal convoluted tubules in 26-day- old control rabbit embryo showing nucleus (N), vacuoles (V), mitochondria(arrows) and microvilli(MV). X 2700

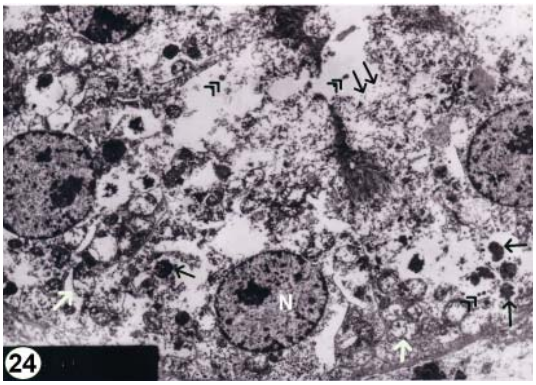

Fig. 24: An electron micrograph of degenerated cells of the proximal convoluted tubules in 26-day- old lead treated rabbit embryo showing nucleus (N), lost microvilli (double arrows), metal deposits (double arrowheads), intracytoplasmic inclusion bodies (black arrows) and swollen degenerated mitochondria (white arrows). X 2700

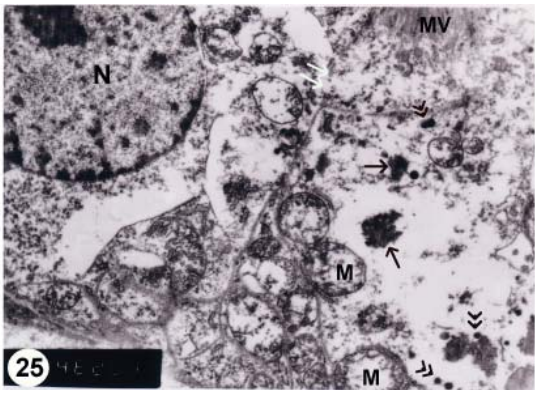

Fig. 25: An electron micrograph of degenerated cells of the proximal convoluted tubule in 26-day- old lead treated rabbit embryo showing nucleus (N), less developed microvilli (MV), metal deposits (double arrowheads), intracytoplasmic inclusion bodies (black arrows) and swollen degenerated mitochondria (M). Note loss of intercellular junction (white arrows). X6700

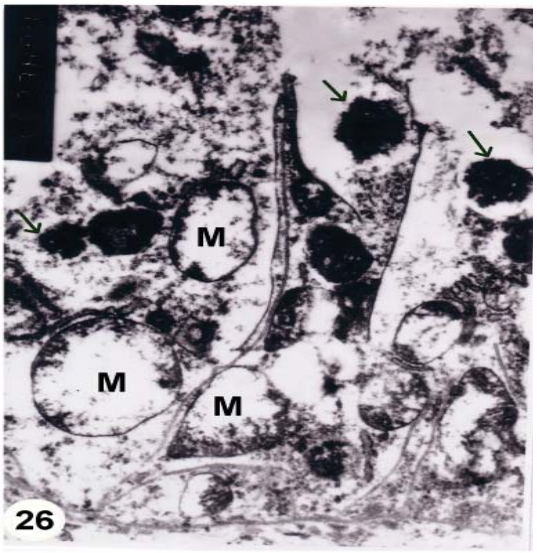

Fig. 26: An electron micrograph of the basal portion of a cell of the proximal convoluted tubule in 26-day- old lead treated rabbit embryo showing intracytoplasmic inclusion bodies (black arrows) and swollen degenerated mitochondria (M). X 8000

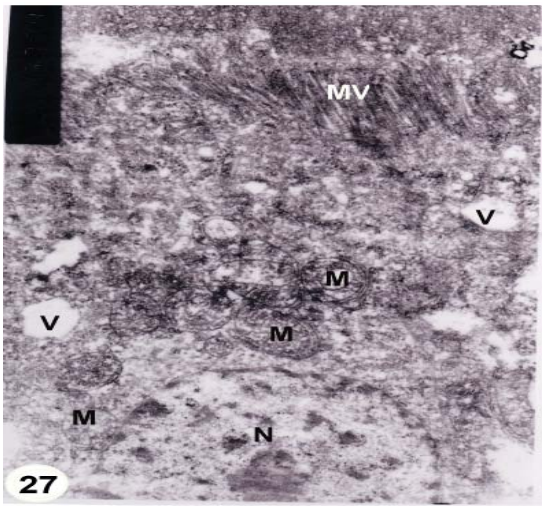

Fig. 27: An electron micrograph of a cell of the proximal convoluted tubule in control newborn rabbit showing euchromatic nucleus (N), vacuoles (V), mitochondria(M) and microvilli(MV). X 6700

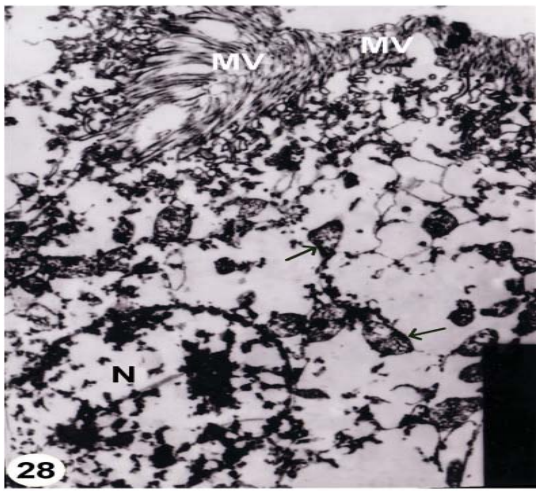

Fig. 28: An electron micrograph of markedly degenerated cell of the proximal convoluted tubule in lead treated newborn rabbit showing nucleus (N) with clumped chromatin, disfigured mitochondria (arrows) and loss of microvilli (MV). X 6700

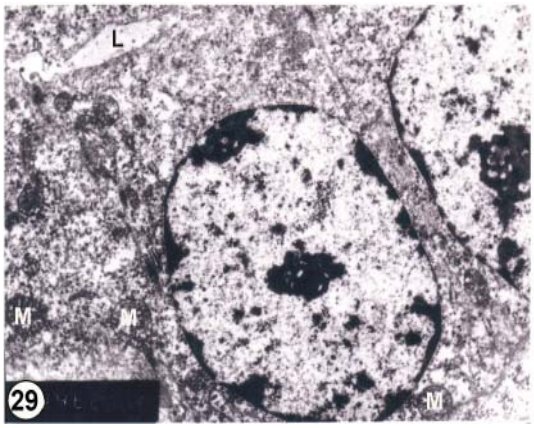

Fig. 29: An electron micrograph of cells of the distal convoluted tubule in 26-day- old control rabbit embryo showing large nucleus and mitochondria (M).Note lumen of the tubule (L). X 6700

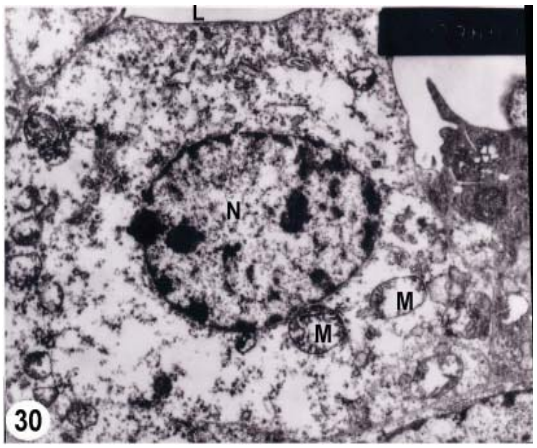

Fig. 30: An electron micrograph of a degenerated cell of the distal convoluted tubule in 26-day- old lead treated rabbit embryo showing vacuolated cytoplasm, degenerated mitochondria (M) and intranuclear inclusion bodies. Note lumen of the tubule (L), nucleus (N) and a large inclusion body entering the nucleus at 9o'clock and a smaller one above it invaginating the nucleus. X 6700

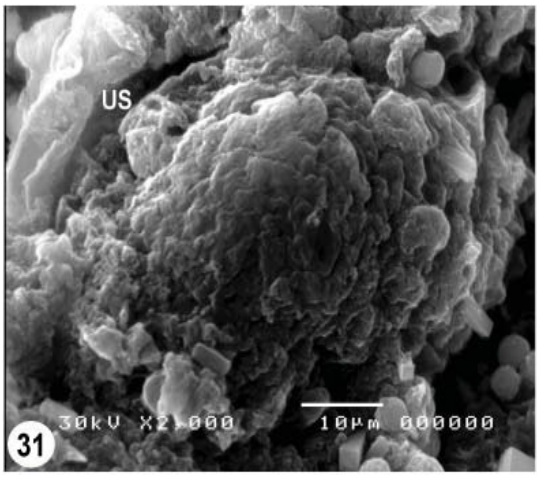

Fig. 31: A scanning electron micrograph of the renal corpuscle in the metanephros of control newborn rabbit showing the glomerulus and the glomerular space (US) . X 2000

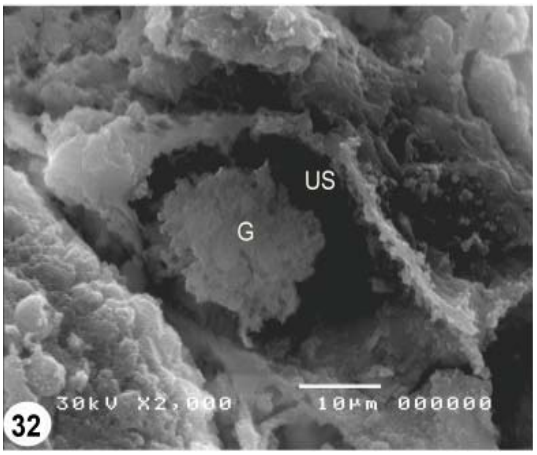

Fig. 32: A scanning electron micrograph of the renal corpuscle in the metanephros of lead treated newborn rabbit showing atrophic glomerulus (G) with wide glomerular space (US) . X 2000

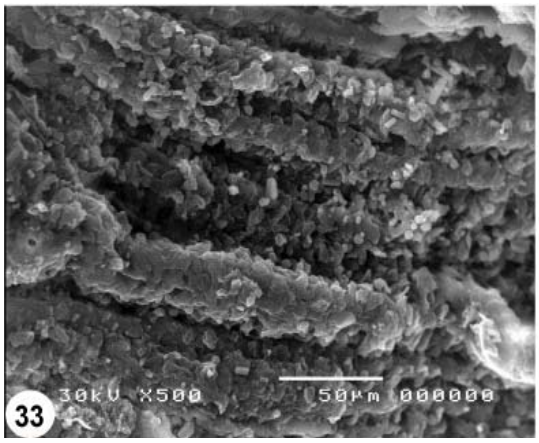

Fig. 33: A scanning electron micrograph of the proximal convoluted tubules in the metanephros of control newborn rabbit showing the interdigitated microprojections on their lateral surfaces. X 500

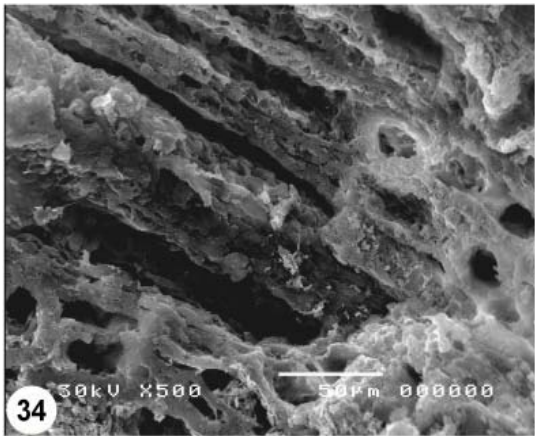

Fig. 34: A scanning electron micrograph of the proximal convoluted tubules in the metanephros of lead treated newborn rabbit showing massive degeneration of their lateral surfaces. X 500

DISCUSSION

In this work, rabbit was selected as a model because fetuses were large enough to handle at early stages of renal development and the kidneys of rabbit pups exhibit anatomic and functional characteristics similar to those of human neonatal kidneys *(McVary and Maizels, 1989)*.

At the age of 16- day- old control rabbit embryo, the present work showed that the metanephric kidney was apparent in the form of a mass behind the caudal part of the mesonephros. It was formed of metanephric caps around the ampullary terminal ends of the ureteric bud. In succeeding ages, the metanephros increased in size with the appearance of nephrogenic zone containing different stages of nephron development. The nephrogenic zone persisted until birth. These results are in harmony with those of *Saxén (1987)*, *Saxén and Sariola (1987)*, *McVary and Maizels (1989)* and *Dressler (2006)*. In addition, *Lechner and Dressler (1997)*, *Sariola and Sainio (1997)* and *Vainio et al. (1999)* reported that the reciprocal tissue interactions between the ureteric bud and the mesenchyme play a key role throughout the period of nephrogenesis process. This complex process of nephrogenesis apparently involves sequentially activated genes and both contact-mediated and secreted signals *(Dressler, 2006)*.

Several lines of evidence, mostly derived from animal studies, indicate that changes in fetal environment may affect renal development *(Williams and Williams, 1990)*. Studies of *Vyskočil et al. (1995a)* suggested that exposure to lead starting at weaning was more renotoxic than exposure starting 2 months later. However, prenatal exposure might also have been a contributory factor. In lead treated animals, the present work showed dispersion and disorganization of the cells of the metanephros at the age of 16 days. This disorganization continued in the formed nephrogenic zone in the succeeding ages. In additions, cavitations and increased interstitial areas were observed. In accordance with these results, interstitial fibrosis was observed in the adult renal tissue of men and animals exposed to subtoxic lead level *(Goyer, 1989; Massanyi, et al. 2007)*. Moreover, *Goyer and Rhyne (1973)* as well as *Nolan and Shaikh (1992)* described the interstitial fibrosis and scaring as a stage of irreversible lead nephropathy. Contrary to the previous result, *Errede et al. (2001)* observed normal appearance of the embryonic metanephros of chick embryo exposed to lead on the light microscopic level. This may be due to short duration of exposure, which was three days.

The present work revealed the presence of dilated vascular spaces in the interstitium of the metanephros at the age of 20 day-old- embryo and in the succeeding ages. This result coincides with the results reported by *Massanyi et al. (2007)* and *Prozialeck et al. (2008)*. The latter authors stated that the vascular system is a critical target of metal toxicity, including lead and perinatal exposure to lead may cause sustained elevations in adult blood pressure. Moreover, actions of metals (lead) on the vascular system may play important roles in mediating the pathophysiologic effects of metals in specific target organs.

The present light microscopic study showed gradual degeneration of the proximal convoluted

tubules, discontinuity of the brush border and vacuolations of the epithelial cells with cystic changes. This marked injury of the epithelial cells of the proximal convoluted tubules was apparent by the electron microscopic study of the present work. These results are in agreement with those of *Hass et al. (1964)*, *Spit et al. (1981)* and *Durgut et al. (2008)* in rabbits, *Vyskočil et al. (1995b)*, *Oberley et al. (1995)* and *Jarrar (2001)* in rats and *Papaioannou et al. (1998)* in dogs. In addition, *EL-Safty et al. (2004)* reported that lead exposure in Egyptian workers caused damage to the renal proximal tubules and resulted in their dysfunction. This metal interacts with renal membranes and enzymes and disrupts energy production, calcium metabolism, glucose homeostasis, ion transport processes and the rennin- angiotensin system *(Nolan and Shaikh, 1992)*.

The electron microscopic study of the present work revealed the presence of metal deposits within the cells of the proximal convoluted tubules. In agreement with these results, *Errede et al. (2001)* detected metal deposits in the lumen of the tubular system and within the proximal tubule cells but never in the renal corpuscles. Moreover, *Papaioannou et al. (1998)* observed metal deposits in the interstitial tissues of the kidney. By the time, the metal's movement through the glomerular filter and then its uptake by the PT cells seem to cause damage *(Errede, et al. 2001)*. This is in harmony with the changes observed in the present work which were altered renal corpuscles, suffering PT cells with swollen mitochondria. In addition, *Mehrotra et al. (2008)* found that the kidney accumulated the maximum amount of lead when treated from different doses in a short period. The long-term changes provoked by the lead acetate treatment in the embryonic kidney might be related to the well-known lead interaction with the cell biochemical systems and give further evidence about the lead nephrotoxicity *(Errede, et al. 2001)*. In accord with this finding, *Gonick (2008)* reported that lead accumulation in the proximal tubules lead to hyperuricaemia and gout, presumably by inhibiting uric acid secretion and diminished glomerular filtration rate. Moreover, intracellular lead is associated with specific high affinity for proteins *(Nolan and Shaikh, 1992)*.

In the present work, important ultrastructural features were seen in the mitochondria due to lead intoxication. These were in the form of swelling of these organelles with destruction of their cristae. These results are concordant with those of *Goyer (1968)*, *Goyer and Rhyne (1973)*, *Oskarsson and Fowler (1985a,b)* and *Jarrar (2001)*. These mitochondrial changes may indicate a special lead affinity for mitochondrial membranes, which play a key role in the functional integrity of these organelles *(Fowler, et al. 1980)*. The mitochondrial swelling reflects injury or impairment of metabolic activity of mitochondrial membranes of the cells *(Goyer, 1968)*. These injured cells were unable to perform efficient functions especially the oxidative phosphorylation and ATP production *(Goyer, 1968; Jarrar, 2001)*. Moreover, the decrease in the specific activities of mitochondrial-based heme pathway enzymes has also been reported *(Fowler, et al. 1980; Oskarsson and Fowler, 1985a; Fowler, 1993)*. Overall, it is clear that this organelle system is a highly sensitive, early target for lead in the kidney.

The present study revealed the presence of inclusion bodies in the cells of the proximal and distal convoluted tubules. These results are in harmony with those of *Goyer (1968)*, *Goyer and Rhyne (1973)*, *Vicente-Ortega et al. (1996)* and *Gonick (2008)* who described those bodies as intranuclear while they were intracytoplasmic as revealed in the present work. However, *Goyer (1982)* postulated that the inclusions were formed in the cytoplasm then migrated intranuclear. This observation was evident in the present work. Lead-induced inclusion bodies have been shown by a number of techniques to be composed of a lead-protein complex *(Carroll, et al. 1970; Goyer et al., 1970b)*. *Goyer and Rhyne (1973)* as well as *Moore and Goyer (1974)* suggested that the inclusion body serves as an adaptive or protective mechanism during transcellular transport of lead as lead within the inclusion bodies is 60-100 times more concentrated than in the whole kidney *(Goyer, et al. 1970a)*. Studies of *Fowler and DuVal (1991)* have identified several high-affinity lead-binding proteins (PbBP) from rat kidney that appear to act as receptors for lead and mediate its activity within these target tissues. This leads to novel alterations in renal gene expression associated with lead uptake into the nucleus *(Fowler, et al. 1985; Fowler, 1998)*. It is further hypothesized that some of these novel gene products may be oncogenes whose expression may account for the carcinogenic effects of lead *(Fowler and DuVal, 1991; Fowler, 1993)*.

Contrary to that result, many workers did not observe inclusion bodies in the cells of the proximal convoluted tubules *(Spit, et al. 1981; Papaioannou, et al. 1998; Durgut, et al. 2008)*. The variations in the presence or absence of inclusion bodies may be due to the difference in the physiology of the animal species, the dose of lead or the route and duration of administration.

Scanning electron microscopy of the present work showed marked shrinkage of the glomerular tuft with an increase in the glomerular space and marked degeneration of the tubules. In accordance with the present study, *Errede et al. (2001)* observed mild changes in some glomeruli in the form of reduced glomerular size with an increase in the glomerular space. This might be due to very short duration of the lead acetate or due to the different species from that of the present work.

The present morphometric study, at the age of 24-prenatal days and the age of newborn lead-treated animals, showed significant reduction in the number of glomeruli per mm, significant reduction in the glomerular diameter with significantly widened glomerular space in comparison with the control animals. This indicated shrinkage in the glomerular tuft of the lead-treated animals. Contrary to the previous results, *Massanyi et al. (2007)* reported significant increase in the diameter of glomeruli and diameter of Bowman's capsule with an intraperitoneal single dose of high lead concentration in adult rats.

In addition, the present work revealed significant reduction in the number of tubules per mm and the tubular diameter of the lead-treated animals in comparison with the control animals.

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دراسة هستولوجية على تأثير خلات الرصاص على الطور النهائي للكلية الجنينية في الأرنب

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ملخص البحث

أجريت هذه الدراسة لتوضح تأثير خلات الرصاص علي تطور الكلية الخلفية في الأرنب. استخدم في هذا البحث عدد ٦٠ من الأجنة والأرانب حديثي الولادة، حيث قسمت إلى مجموعتين، مجموعة ضابطة وأخرى تجريبية. أعطيت الأمهات الحوامل في المجموعة التجريبية خلات الرصاص بجرعة١٥ مجم /كجم عن طريق الفم من اليوم العاشر من الحمل وحتى الولادة وكذلك أعطيت الأمهات الحوامل في المجموعة الضابطة خلات الصوديوم بنفس النظام. هذا وقد جهزت العينات عند أعمار ١٦، ٢٠ و٢٤ يوما قبل الولادة وكذلك عمر حديث الولادة وصبغت بصبغة الهيماتوكسلين والأيوسين وصبغة البيريوديك أسيد شيف هذا وقد جهزت عينات أخرى عند أعمار ٢٦ يوما قبل الولادة وكذلك عمر حديث الولادة لعمل قطاعات نصف رقيقة و قطاعات فائقة الرقة للفحص الإلكتروني المجهري و للفحص بالميكروسكوب الإلكتروني الماسح وقد أجريت أيضا دراسة مورفومترية اشتملت على قياس أقطار و عدد الكبيبات والأنابيب الكلوية و كذلك قياس الحيز البولي للكبيبة.

أوضحت نتائج هذا البحث ظهور الكلية الخلفية في صورة كتلة محددة خلف الكلية الوسطى عند عمر ستة عشر يوما قبل الولادة. و عند عمر عشرين يوما قبل الولادة ظهرت طبقة المكون الكلوي في الكلية الخلفية تحت المحفظة الكلوية و فيها مراحل متعددة من تطور النفرون (الوحدة الكلوية) و في الأعمار المتعاقبة أخذ سمك هذه الطبقة في الازدياد حتى عمر الولادة حيث لا تزال متواجدة.

وفي الحيوانات التجريبية وجد خلل في خلايا الكلية الخلفية وكذلك طبقة المكون الكلوي في الأعمار المتقدمة، و في الأعمار المتعاقبة لوحظ وجود فجوات وفراغات وعائية متسعة وزيادة في الأنسجة الخلالية وكذلك تغيرات انحلالية للكبيبات والأنابيب الكلوية مع اتساع الحيز الكبيبي.

وعند عمر ٢٦ يوم قبل الولادة فقد أظهرت نتائج فحص القطاعات النصف الرقيقة انحلال في خلايا القنوات الملفوفة الدانية للكلية الخلفية مع وجود تجويفات غير منتظمة وحدوث ضرر للحواف الفرشية وكذلك انحلال في خلايا القنوات الملفوفة القاصية. كما لوحظ احتقان في الأوعية الشعرية للكبيبات. هذا وقد زاد هذا الإنحلال عند عمر الولادة.وعند فحص القطاعات فائقة الرقة ،لوحظ حدوث عطب في الخلايا الطلائية للقنوات الملفوفة الدانية وكذلك في الزغابات الدقيقة مع حدوث فجوات للسيتوبلازم وتورم في الحبيبات الخيطية (الميتوكوندريا) وتكسير لنتوءاتها.كما أنه لوحظ وجود أجسام في السيتوبلازم وكذلك رواسب معدنية. هذا وقد زاد هذا الإنحلال عند عمر الولادة.

و بالفحص باستخدام بالميكروسكوب الإلكتروني الماسح، لوحظ وجود انحلال واضح في الأنابيب و الكبيبات مع اتساع في الحيز الكبيبي مقارنة بالمجموعات الضابطة.

وكذلك اوضحت الدراسة المورفومترية وجود نقصا ذا دلالة إحصائية في عدد و أقطار الكبيبات والأنابيب الكلوية مع اتساع في الحيز الكبيبي في الحيوانات المعالجة بالرصاص مقارنة بالمجموعات الضابطة.